Stefan Petrovic
California Institute of Technology

Linker proteins tie the Nuclear Pore together

Linker proteins tie the Nuclear Pore Complex (NPC) together

Cry
Stefan Petrovic
Hoelz lab, Caltech
April 12th-14th, 2021

Acknowledgements

- **Caltech Center for Molecular and Cellular Medicine**
- **Caltech Molecular Observatory**
  - Jens Kaiser
- **Caltech Cryo-EM Facility – Beckman Institute**
- **PNCC Cryo-EM Center**
- **NYSBC-NCCAT Cryo-EM Center**
- **André Hoelz group**
  - Dipanjan Samanta (postdoc)
  - Thibaud Perriches (postdoc)
  - Karsten Thierbach (postdoc)
  - Chris Bley (lab manager)
  - Bonnie Brown (research tech)
  - Taylor Stevens (grad student)
  - Xiaoyu Liu (postdoc)
  - Giovani Tomaleri (rotation)
  - Lucas Schaus (rotation)
  - Jimmy Thai (Amgen scholar)
  - Alex Lyons (Amgen scholar)
  - Aaron Tang (research tech)
- **Thesis committee**
  - Doug Rees (chair)
  - Bil Clemons
  - Grant Jensen
  - André Hoelz (advisor)

NIH National Institutes of Health

Boehringer Ingelheim Fonds
Stiftung für medizinische Grundlagenforschung

SSRL

PACIFIC NORTHWEST Cryo-EM Center

Daniel Mann
Forschungszentrum Juelich

Macromolecular organization of membrane-associated Atg18 oligomers

Macromolecular organization of membrane-associated Atg18 oligomers

DANIEL MANN

FORSCHUNGSZENTRUM JÜLICH

CARSTEN SACHSE LAB

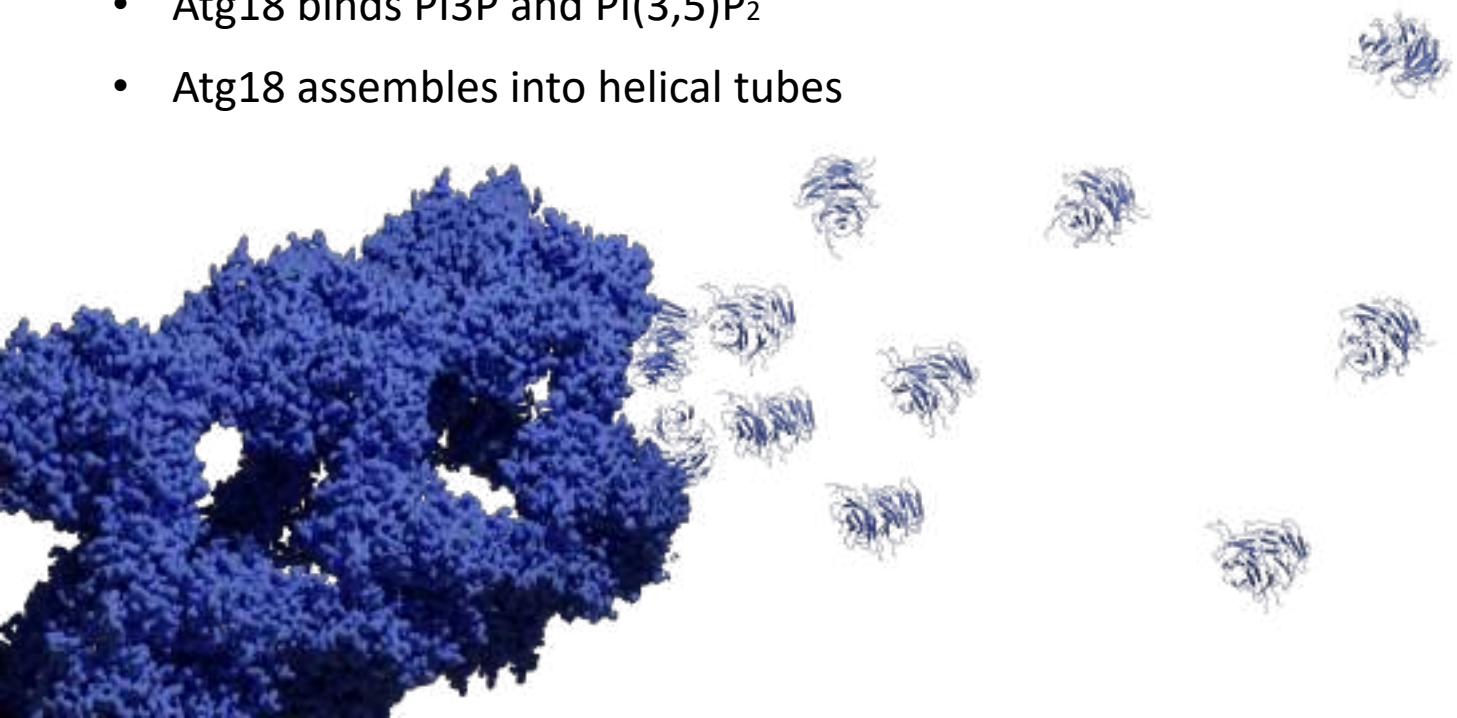
NCCAT TOMO SHORTCOURSE

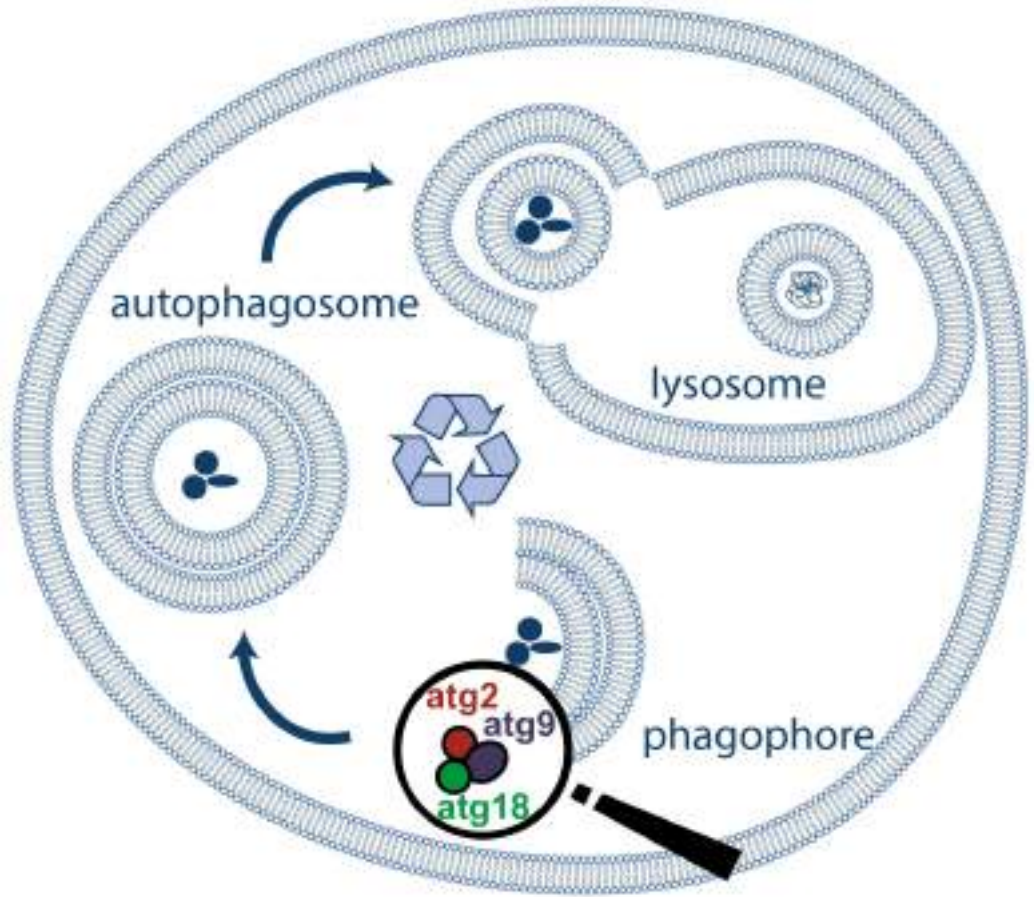
ATG18

A KEY PLAYER IN MACROAUTOPHAGY

- Autophagy
  - Intracellular degradation process

    (recycling and removal of material)
  - Encapsulation, delivery to lysosome
- Atg18: formation and elongation of phagophore
- Atg18 binds PI3P and PI(3,5)P_2
- Atg18 assembles into helical tubes

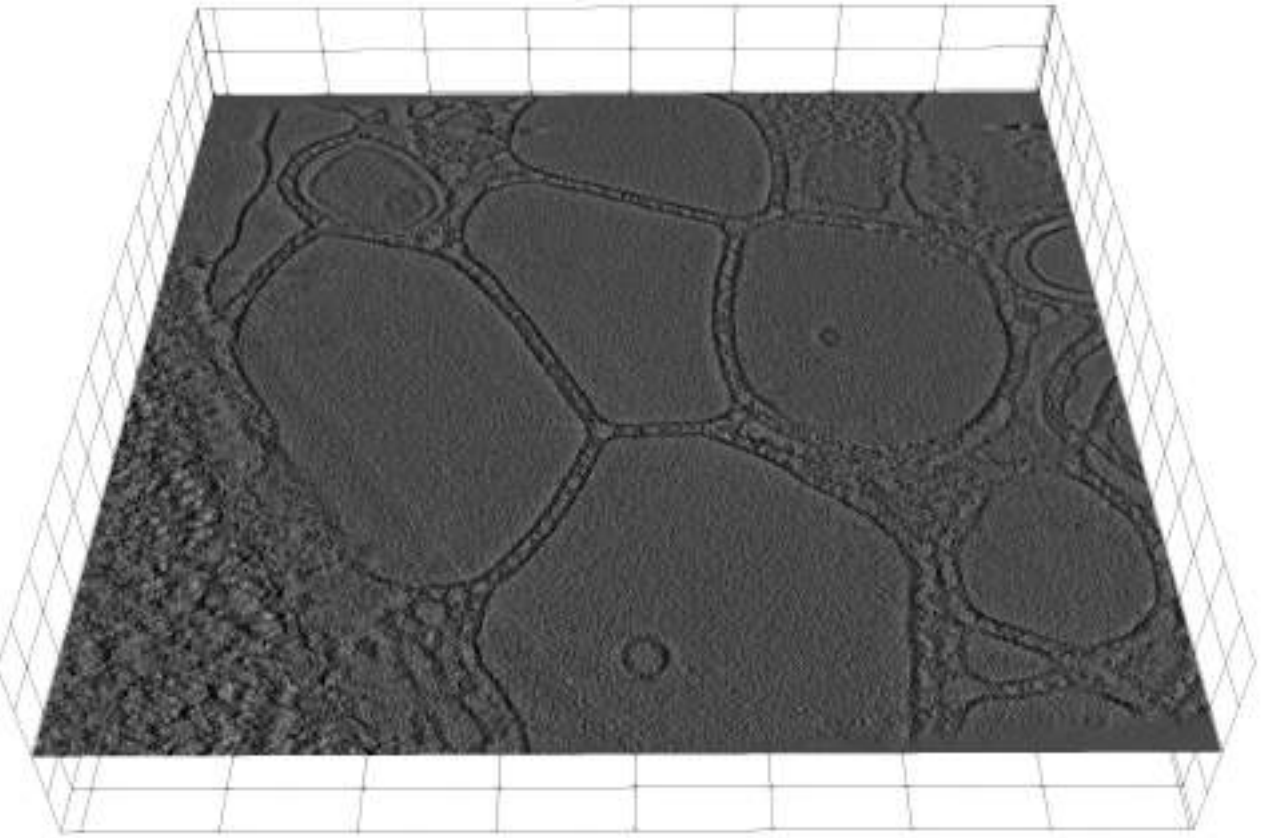




ATG18 on cellular membranes

Tomography of atg18 assemblies

- PySeg package / Relion for subtomo averaging

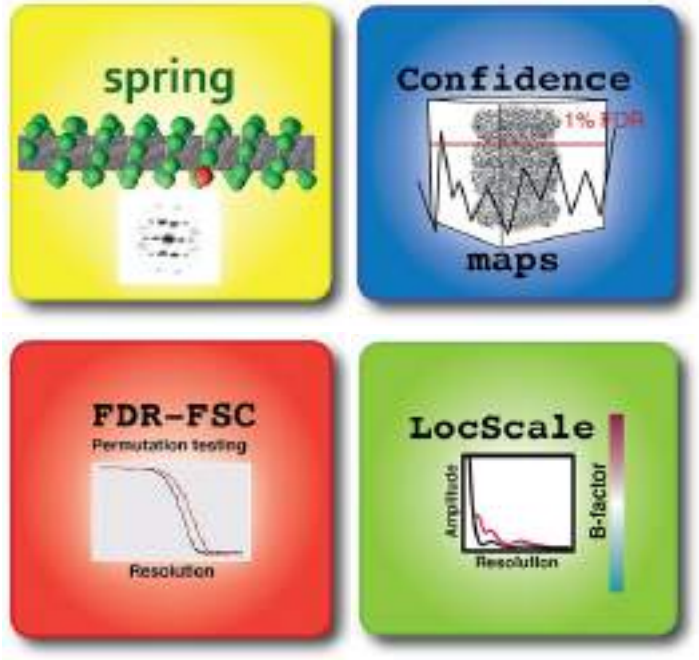


Double membrane binding of a ~50 kDa protein

Martinez-Sanchez et al. *Nat.Meth.* 2020

Ernst Ruska Centre 3 @ Forschungszentrum Jülich

d.mann@fz-juelich.de **www.sachse.fz-juelich.de** **www.fz-juelich.de/er-c/er-c-3**

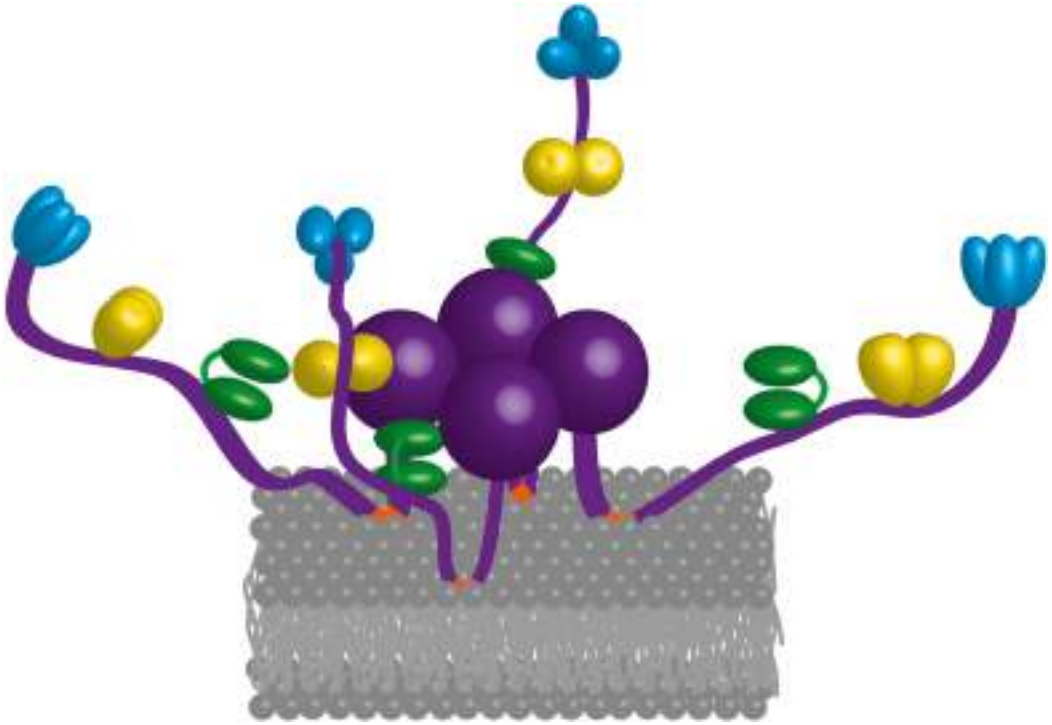


https://erc-3-cloud.fz-juelich.de/FSC_Server/

Giulia Paris
University of Cambridge

Structural studies of the membrane-bound RNA degradosome of E.coli

Structural studies of the membrane-bound RNA degradosome of *E.coli*



Bandrya *et al.,* Biochim Biophys Acta, 2013;

RNA degradosome:

- Multi-enzyme complex involved in mRNA turnover and gene expression regulation
- Bound to the inner membrane of *E.coli*
- Flexible and natively unstructured scaffold domain

UNIVERSITY OF CAMBRIDGE

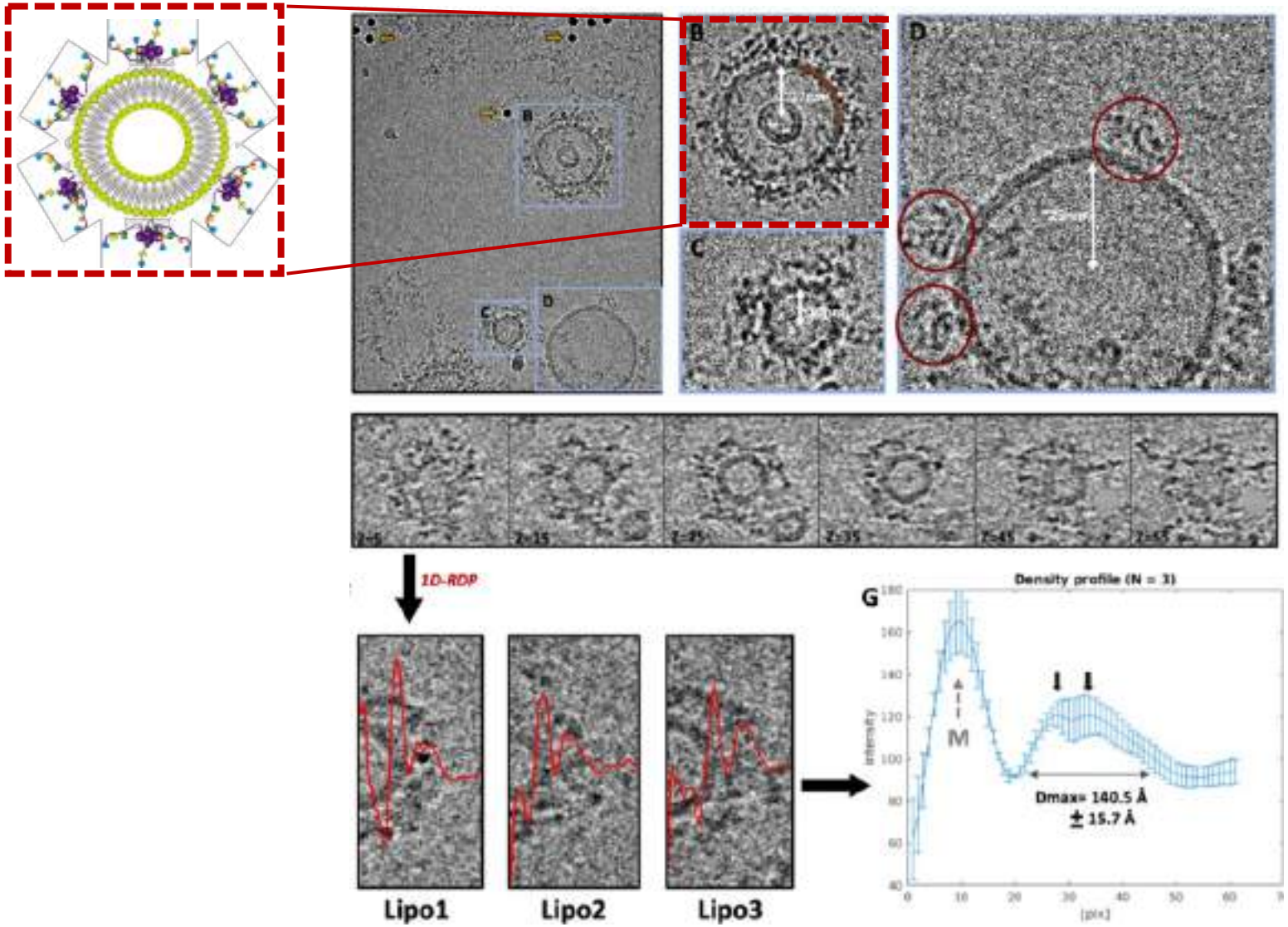
Giulia Paris,
PhD student
Prof. Ben Luisi's group

Biochemistry
University of Cambridge

Structural studies of the membrane-bound RNA degradosome of *E.coli*

- Reconstitution of the complex on membranes
- Cryo-electron tomography
- Measure of the 1D-Radial Density Profile (1D-RDP)

Tom Dendooven

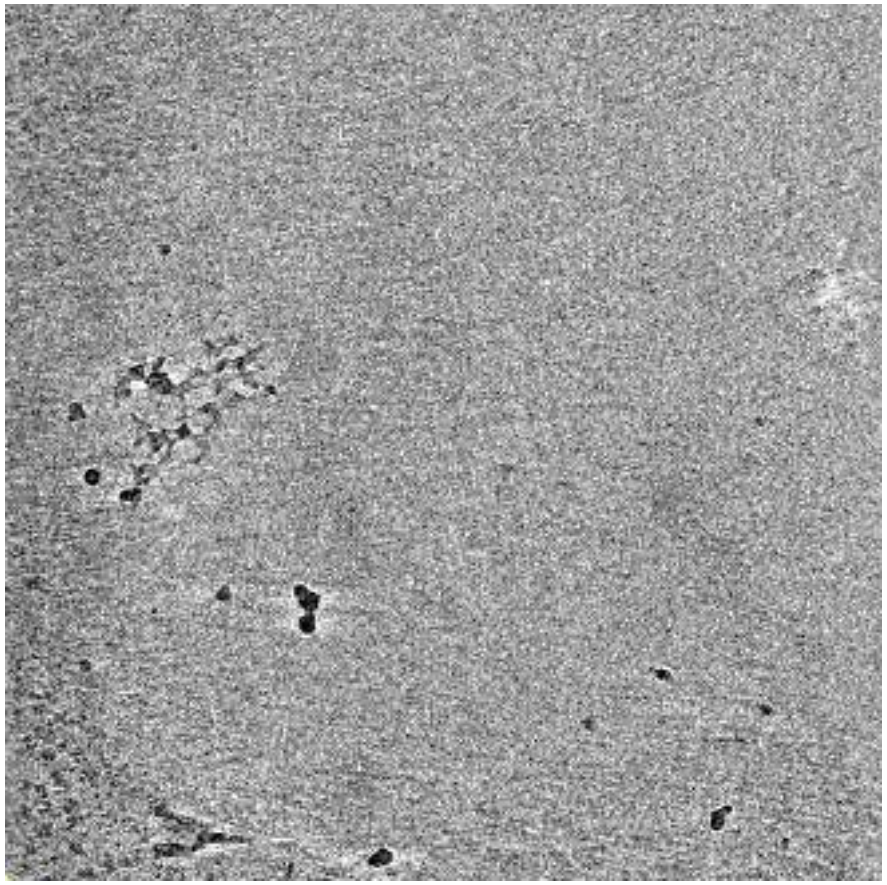


Dendooven, Paris *et al.,* in preparation

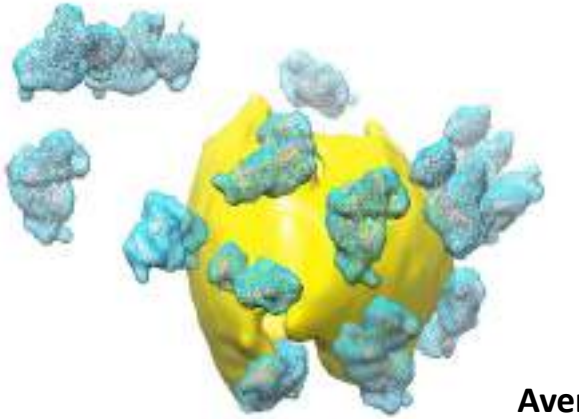
Structural studies of the membrane-bound RNA degradosome of *E.coli*

Interaction with ribosomes: cryo-electron tomography and subtomogram averaging

+ 30S ribosome



emClarity, TomoSegmemTV



Averaged ribosomes mapped back in original tomogram

30S ribosome map – 16 iterations; 9938 particles: 11.7 Å

Nadia Herrera
University of California, San Francisco

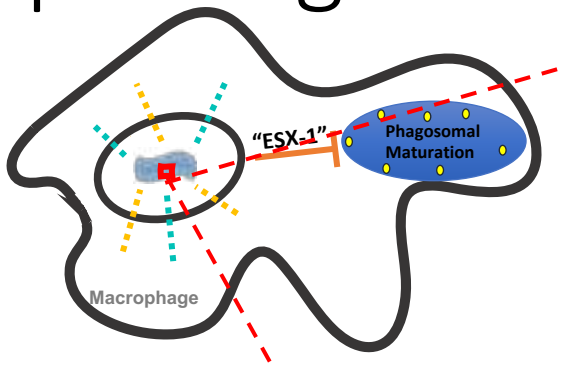
Investigation of the ultrastructural dynamics of the mycobacterial ESX-1 complex

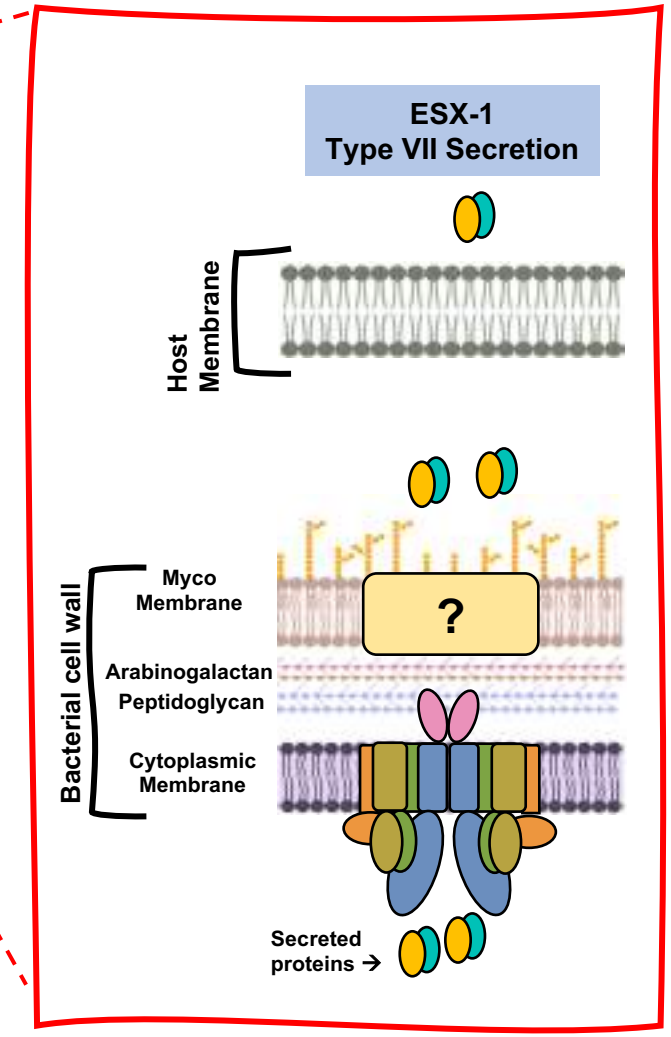
Investigating the ultrastructure of mycobacterial ESX-1 secretion systems

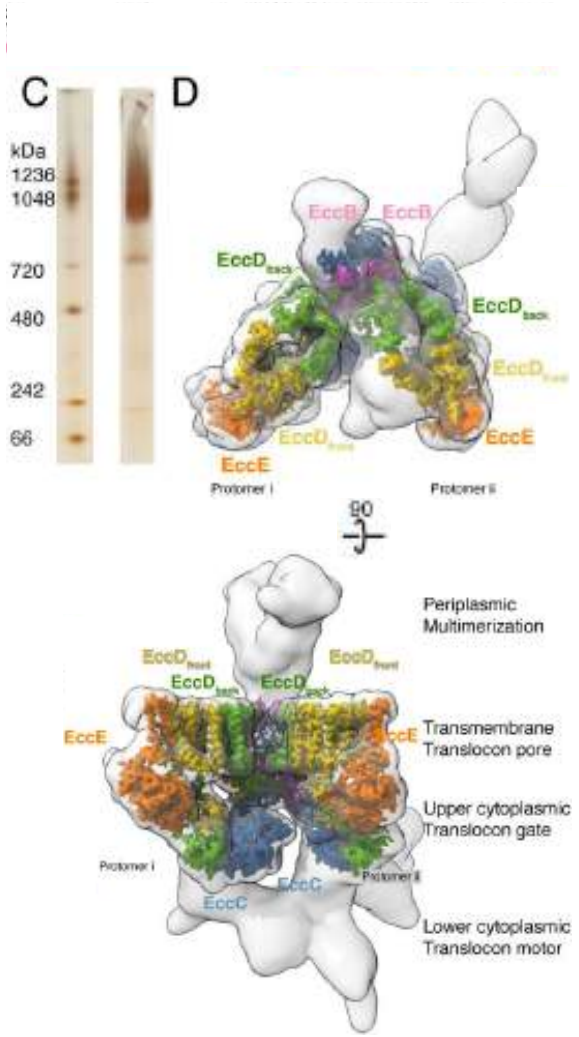
Nadia Herrera, PhD

NCCAT Tomography Short Course
April 12-14, 2021

ESX-1 has been assumed follow paradigm of secretion systems

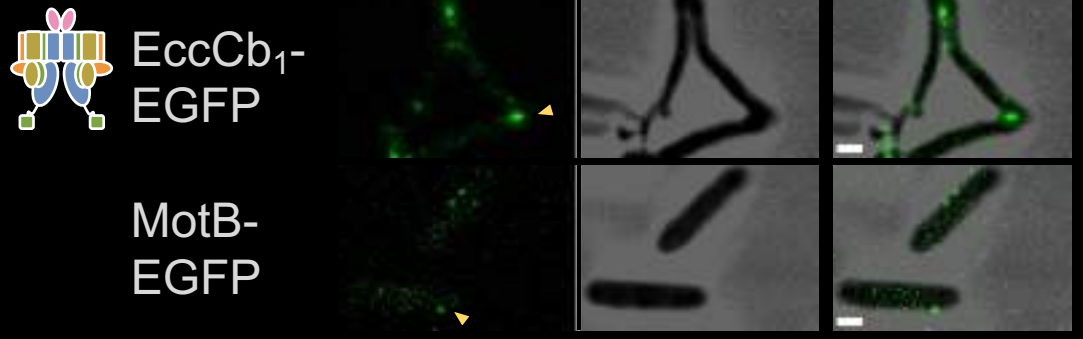


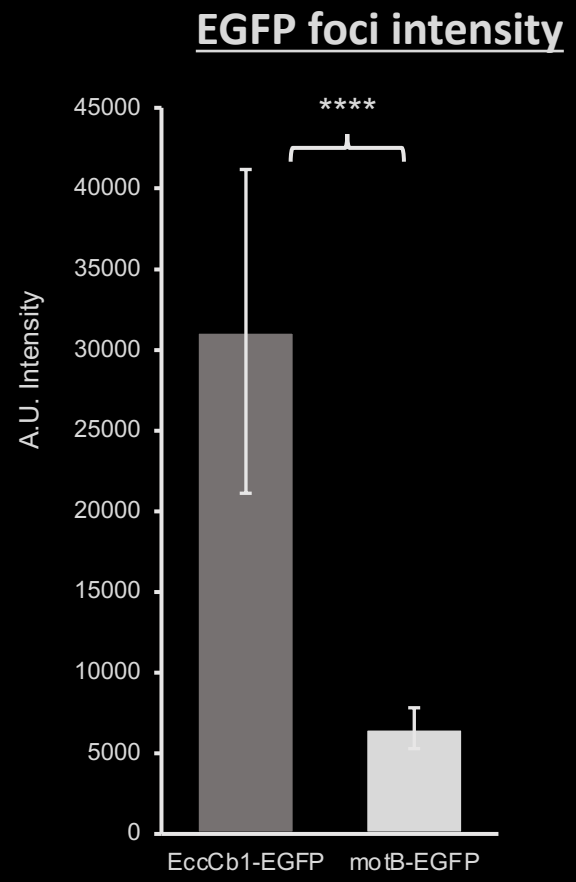




Adapted from:
Poweleit, N., *et al.,* 2019, *eLife.* DOI: 10.7554/eLife.52983

ESX-1 foci in *M. smegmatis* correlate to a large oligomer





Correlates to ~100MDa complex, **~50 ESX-1** complexes.

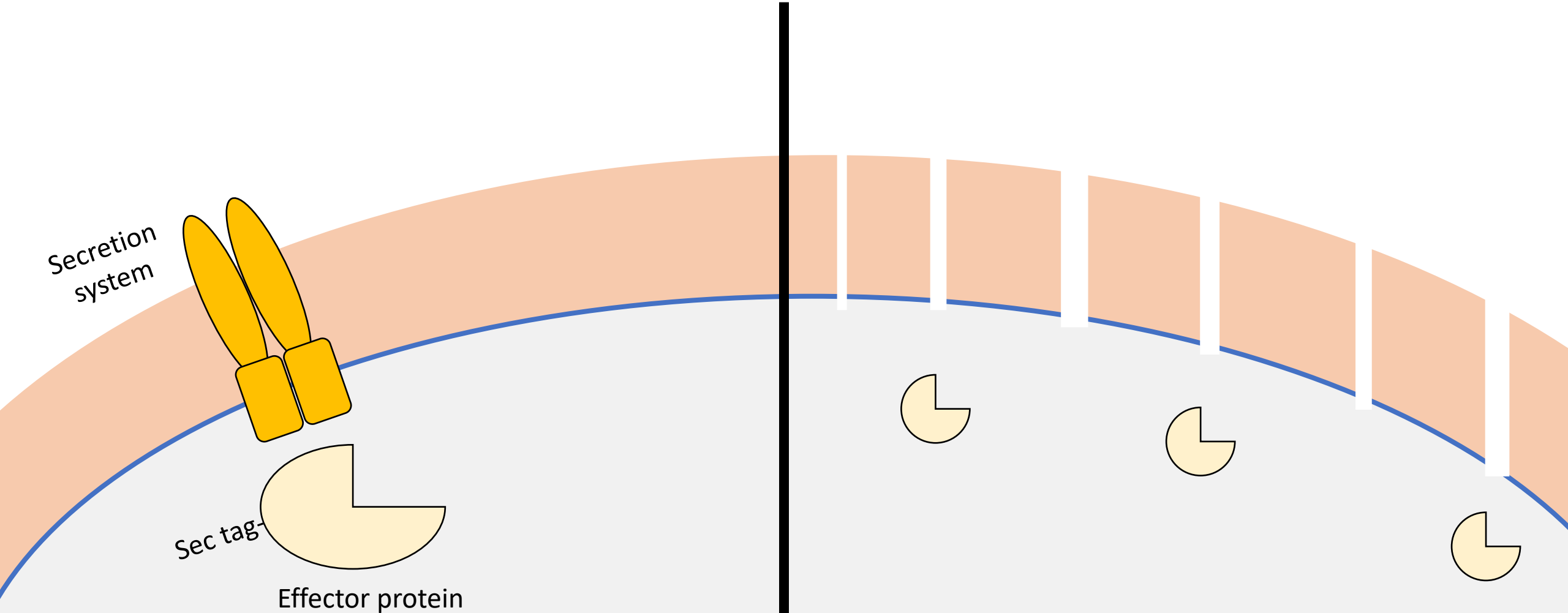
PDBID:6UMM

ESX-1 assembly in whole cells?

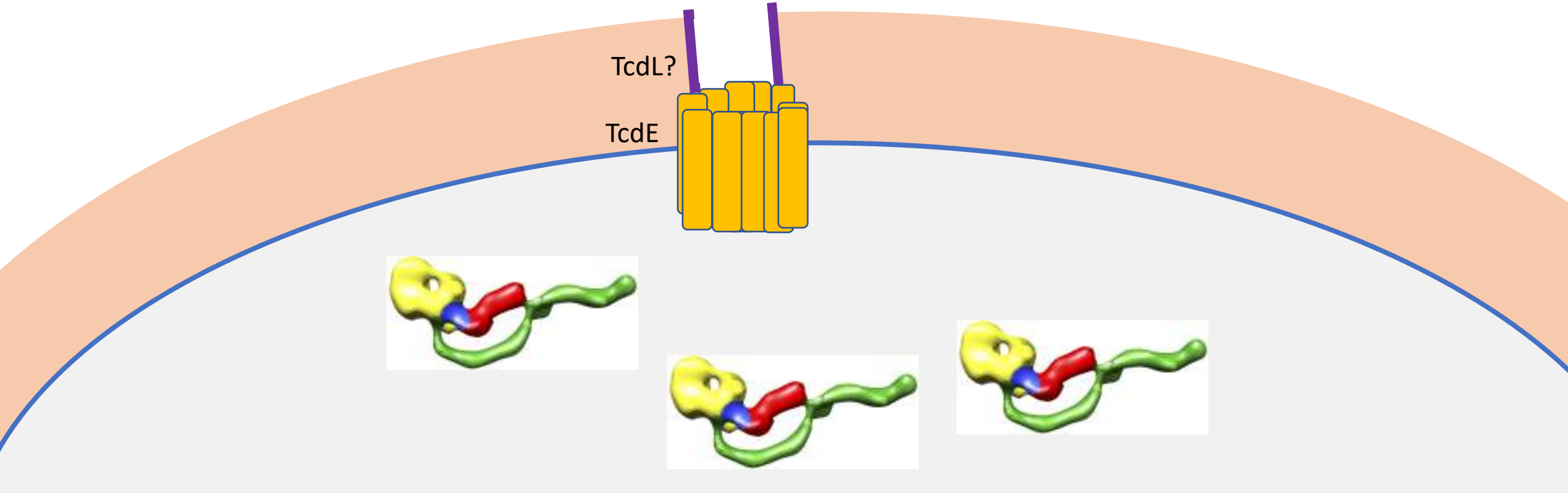
Shannon Kordus
Vanderbilt University Medical Center

Understanding toxin secretion in Clostridioides difficile

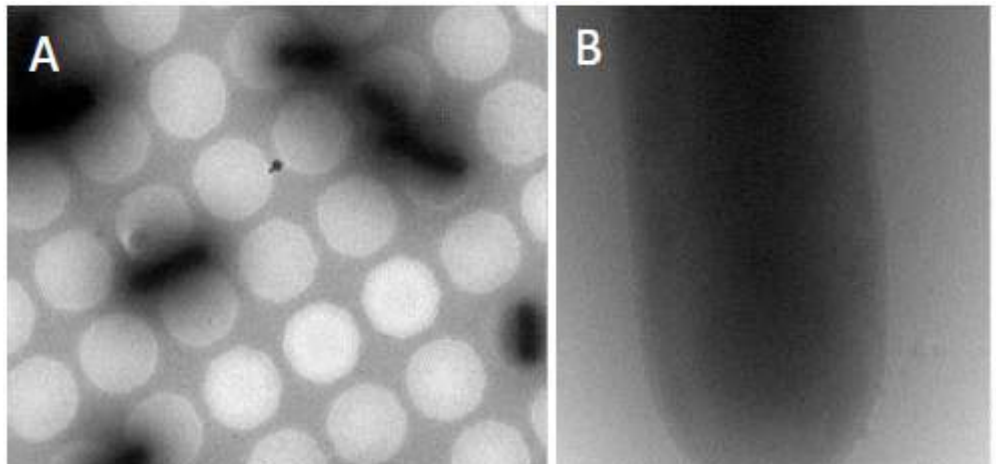
How does a large protein get through the cell wall?

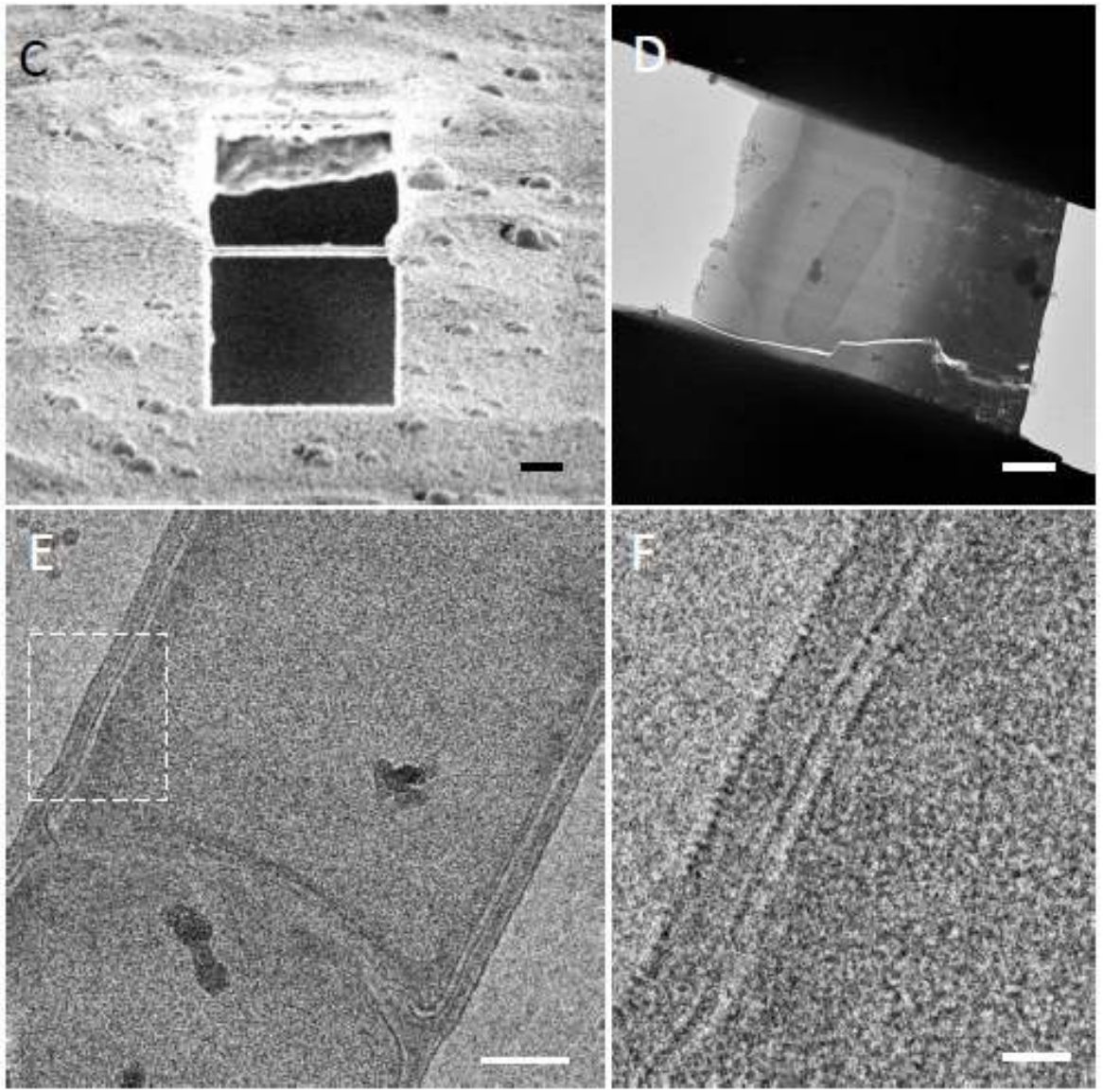


The *Clostridiodes difficile* toxins do not require a secretion tag or cell lysis for secretion



Cryo-ET gives insights into the structure of the *C. difficile* cell wall during toxin secretion





Emily Armbruster
CUNY Advanced Science Research Center

Interactions and Structure of Ryanodine Receptor Clustering on the Sarcoplasmic Reticulum of Cardiac Muscle

Interactions and Structure of Ryanodine Receptor (RyR) Clustering on the Sarcoplasmic Reticulum of Cardiac Muscle

Emily Armbruster, PhD
Postdoc, des George Lab

April 13, 2021

RyR cluster size as well the RyR2 arrangement are dynamic, modulated, and correlated to calcium leaks and heart failure

Cardiac Muscle

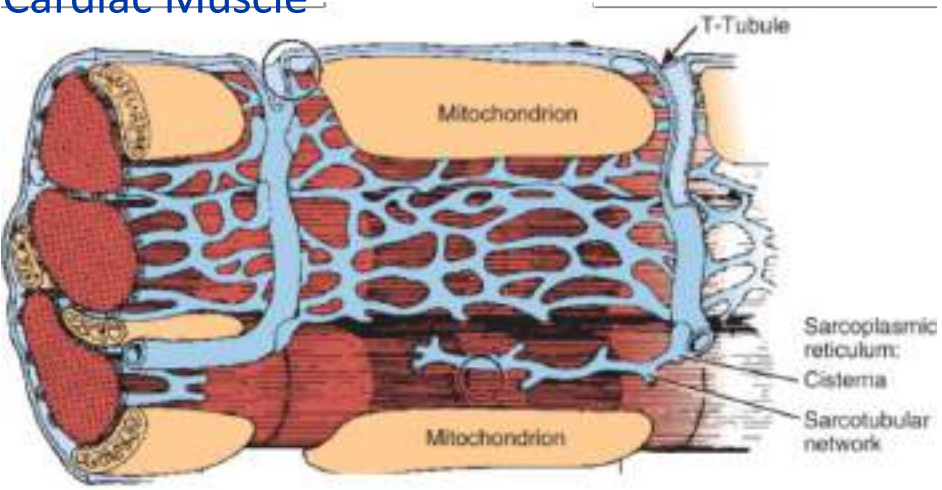


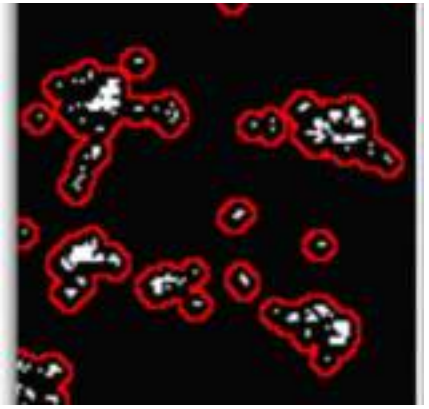
Figure from Katz AM. Physiology of the Heart. 3rd ed. 2001

- Calcium binding one RyR channel can induce multiple RyR channels to open causing calcium spike
- Calcium spike induces muscle contraction
- Clustering of RyRs on membrane impact calcium spike
- Clustering can be modulated by phosphorylation, small molecules, and small accessory proteins

Heart failure correlation

Control heart

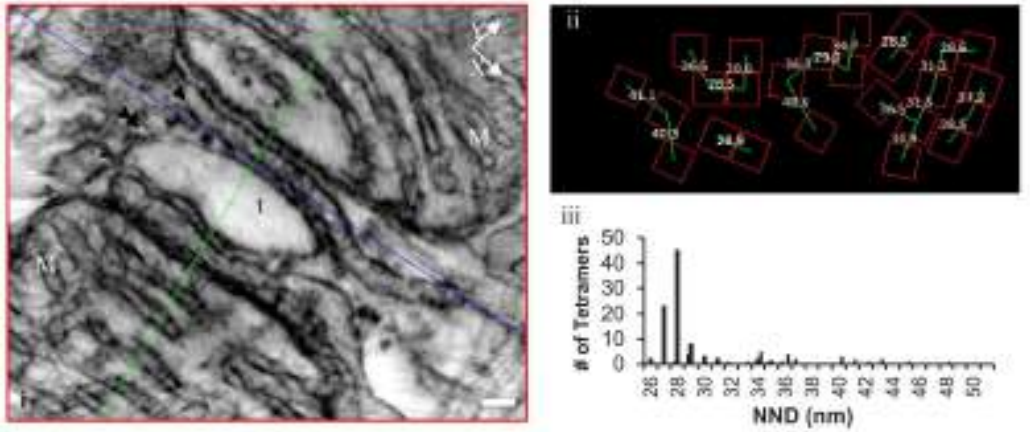
Heart that has failed



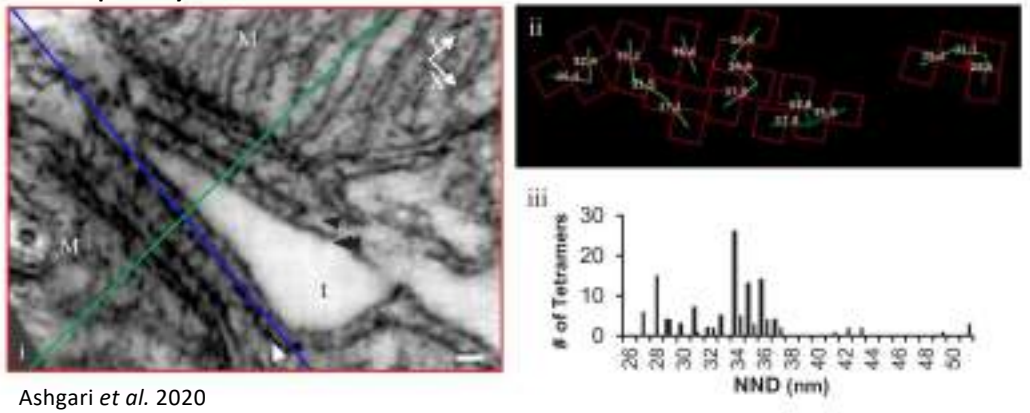
Kolstad *et al.* 2018

Modulation by phosphorylation

Control heart slice

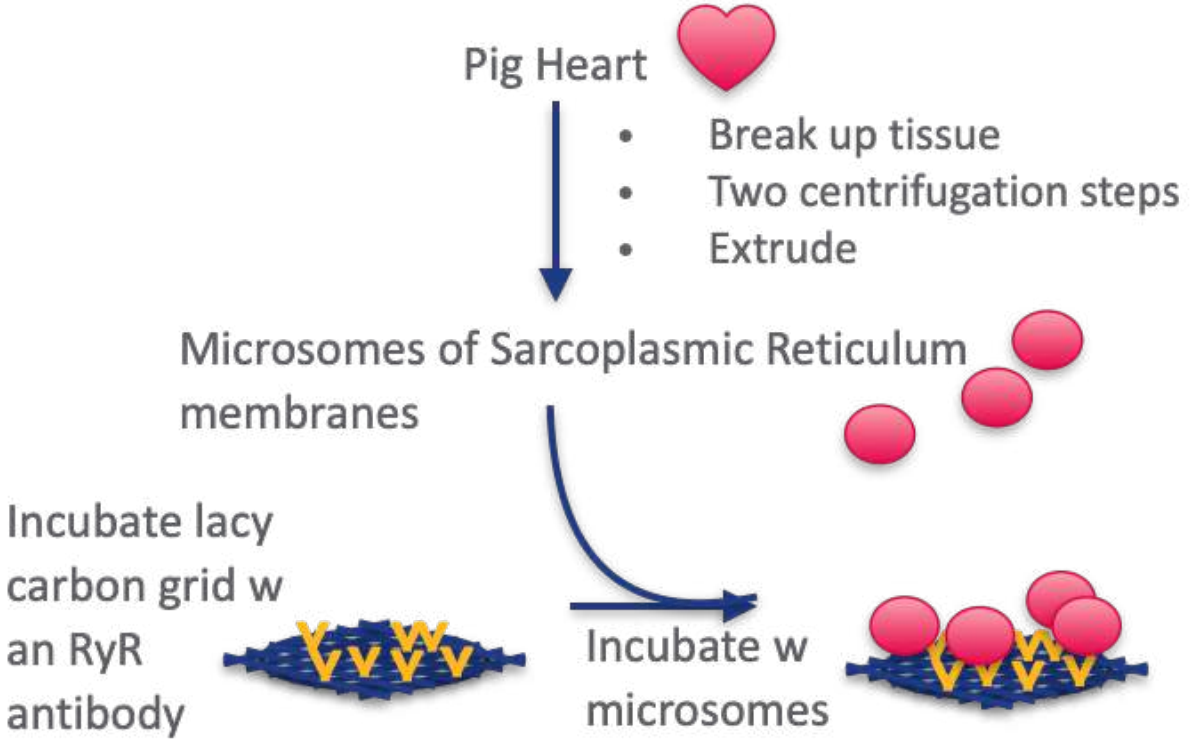


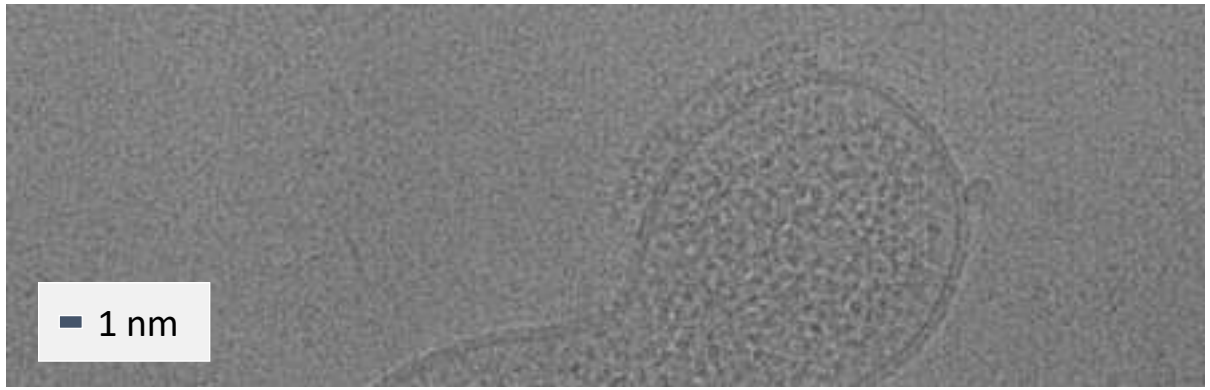
Phosphorylated heart slice

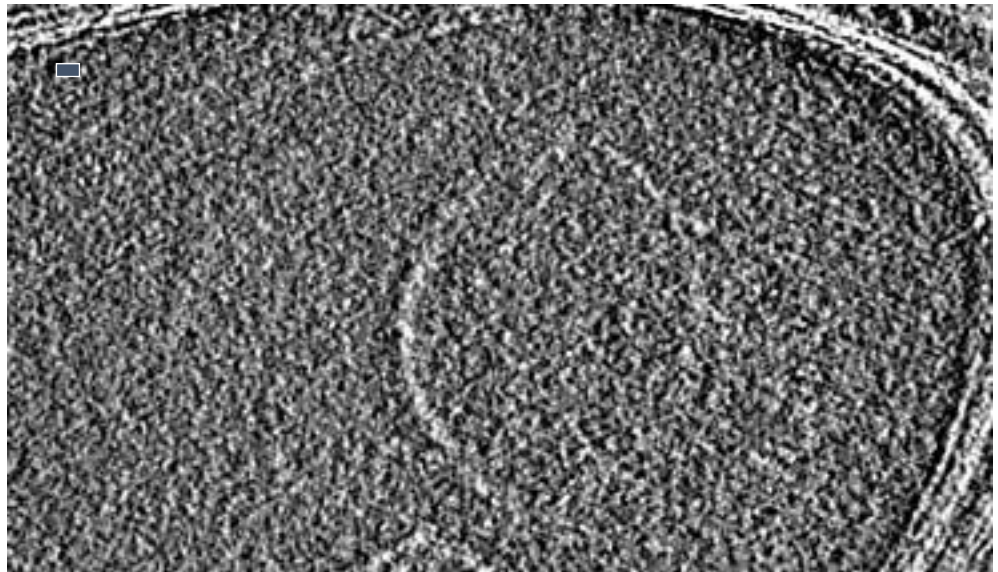


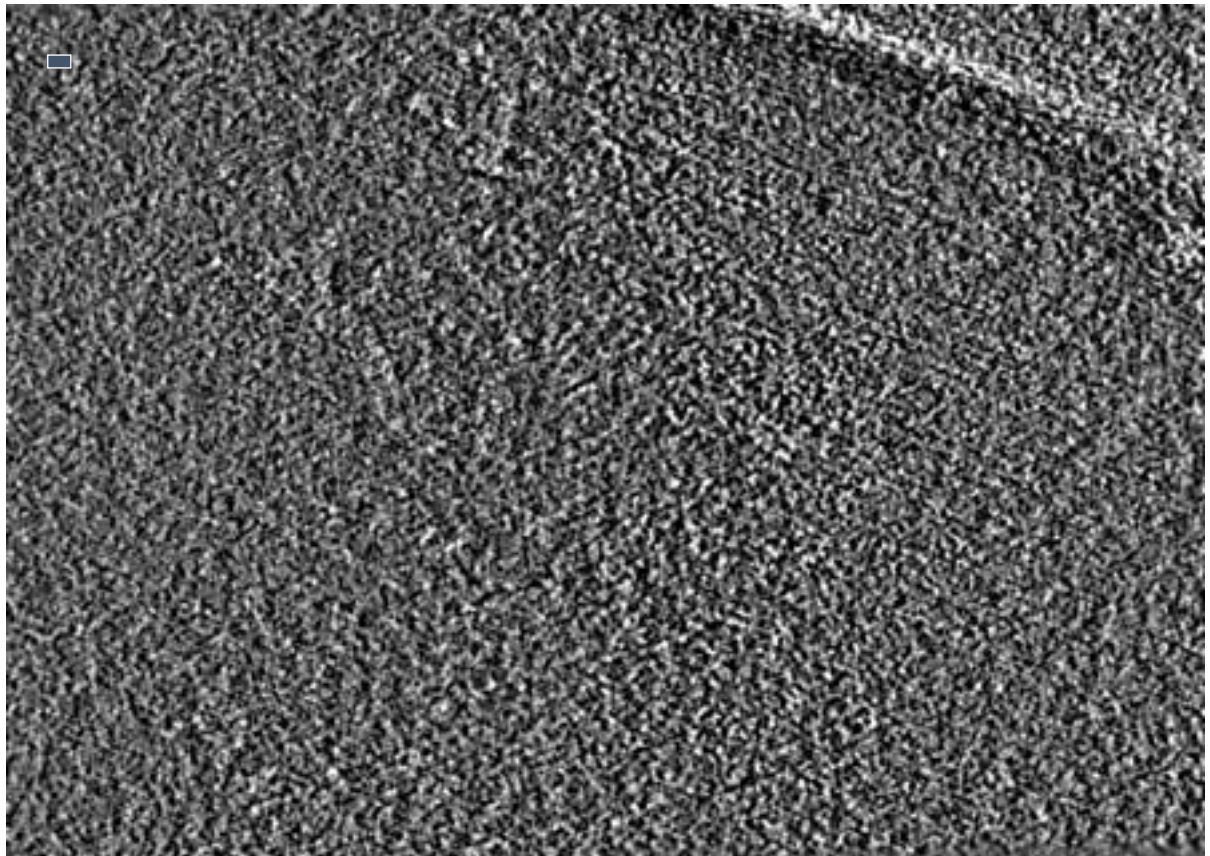
Ashgari *et al.* 2020

Preliminary data of the cardiac SR observed by cryo-ET







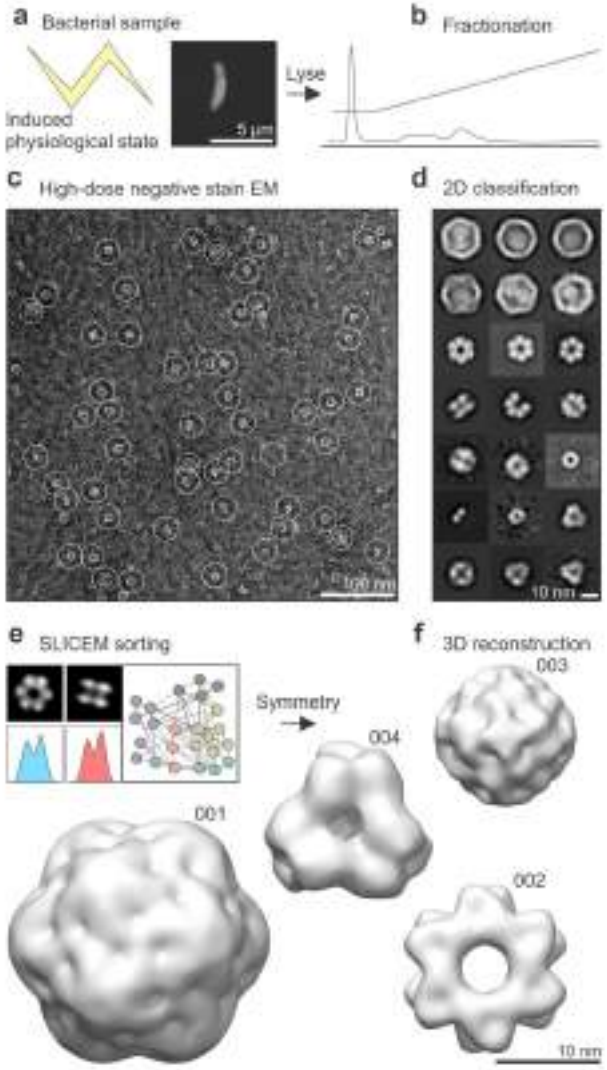


Angela Kirykowicz
University of Cambridge

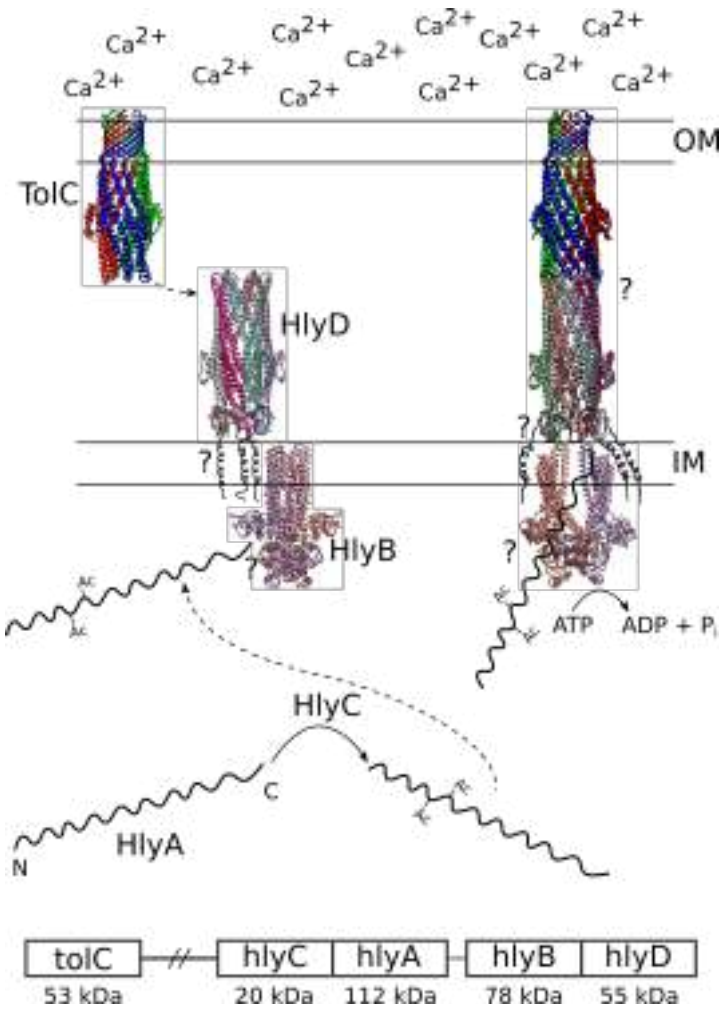
Structure and function of the Type I secretion system transport machine in its cellular context

What Determines Substrate Specificity?

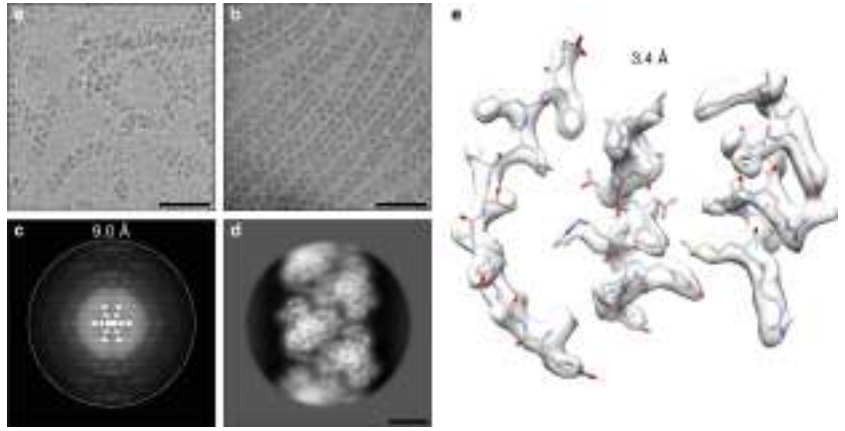
Diversity of quaternary structures in response to stress



Kirykowicz, AM & Woodward, JM. 2020. Shotgun EM of Mycobacterial protein complexes during stationary phase stress. Curr Res Struct Biol. Under Peer Review.



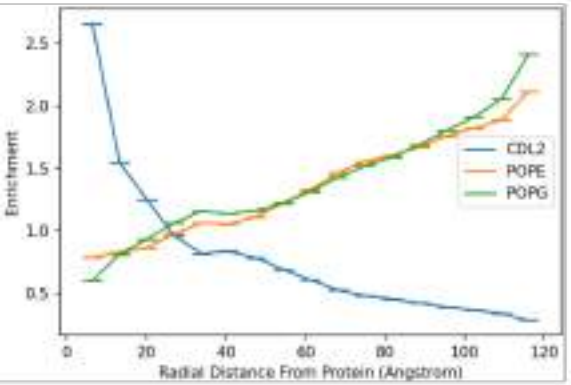
Virulence factor secretion



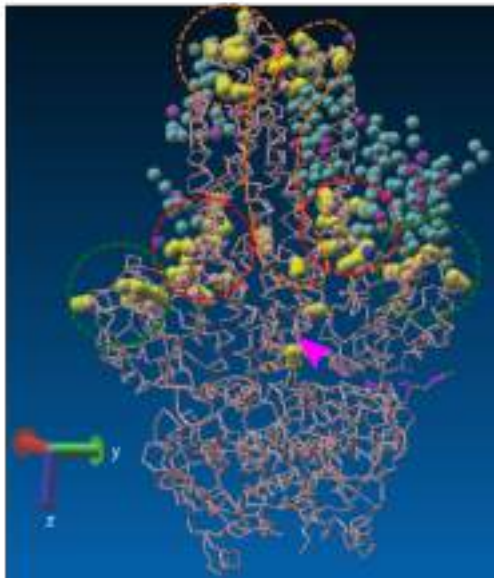
Mulelu, AE, **Kirykowicz, AM**, Woodward, JM. 2019. Cryo-EM and directed evolution reveal how Arabidopsis nitrilase specificity is influenced by its quaternary structure. Commun Biol. 2: 260.

Enzyme substrates

Global

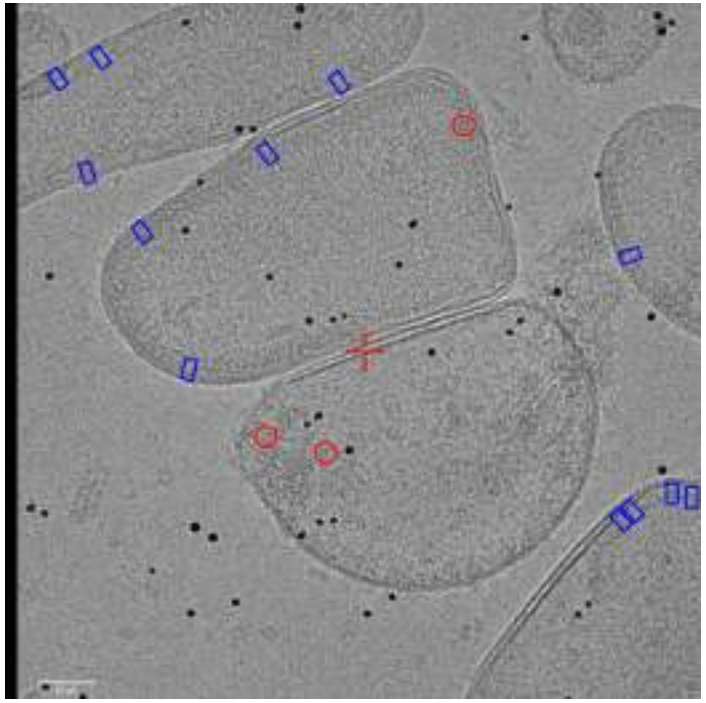


Local

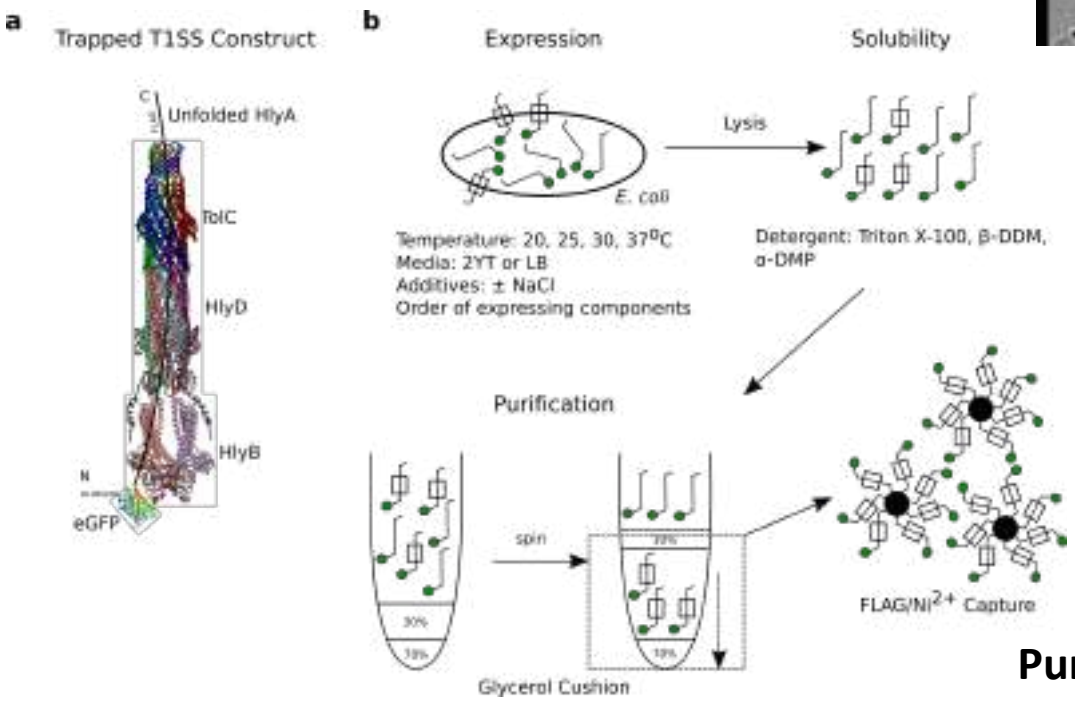


Molecular Dynamics

CryoET



Structure and function of the Type I Secretion System transport machine in its cellular context

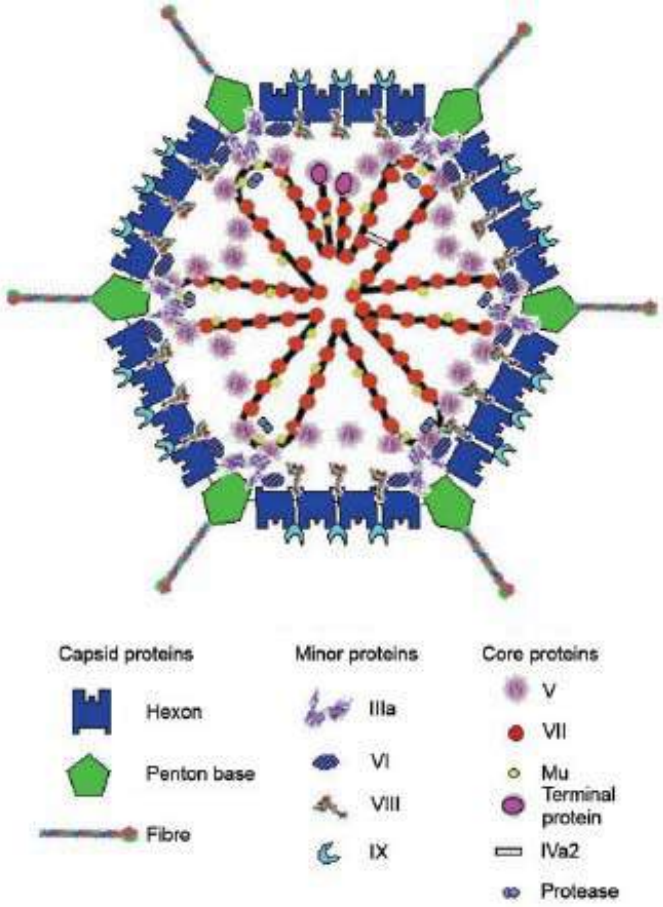


Purification & single-particle cryo-EM

Gabriela Condezo
Spanish National Center for Biotechnology

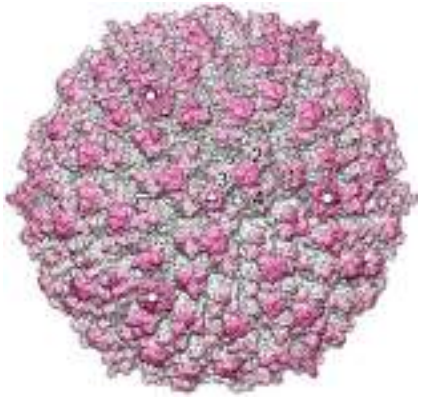
Towards 3D visualization of virus assembly in the cell

Towards 3D visualization of virus assembly in the cell



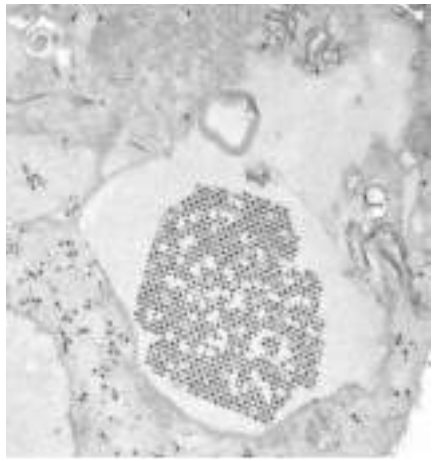
Adenovirus

(Russell WC, 2009. *J Gen Virol 90:1-20).*



Structure of purified viral particles

Marabini et al. 2021
Sci Adv. 7(14)



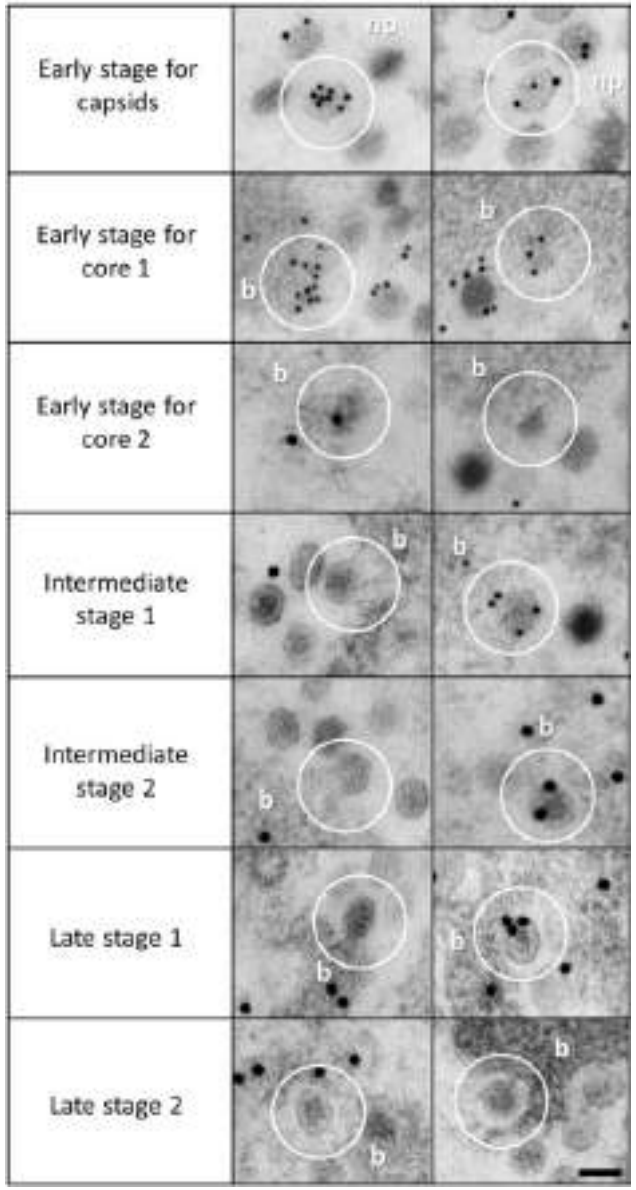
Assembly mechanism

Condezo and San Martín.
2017 PLoS Pathog 27

Gabriela Condezo
Spanish National Biotechnology Centre

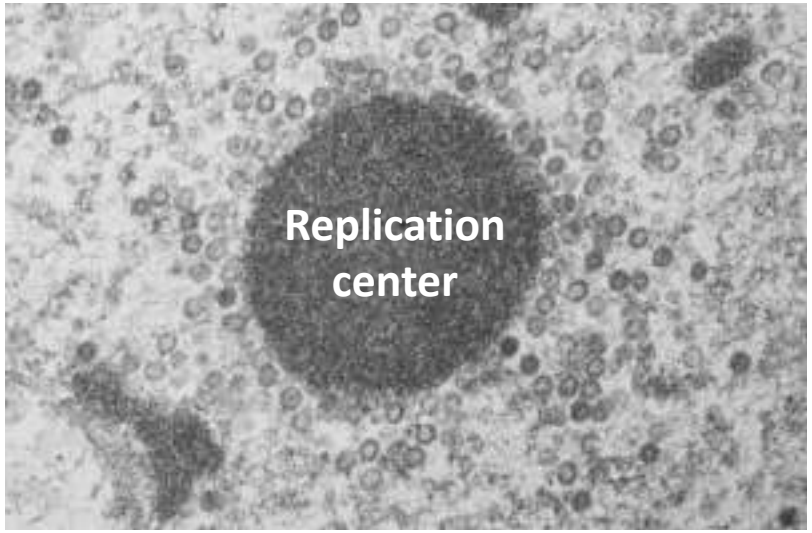
CNB
CENTRO NACIONAL DE BIOTECNOLOGÍA

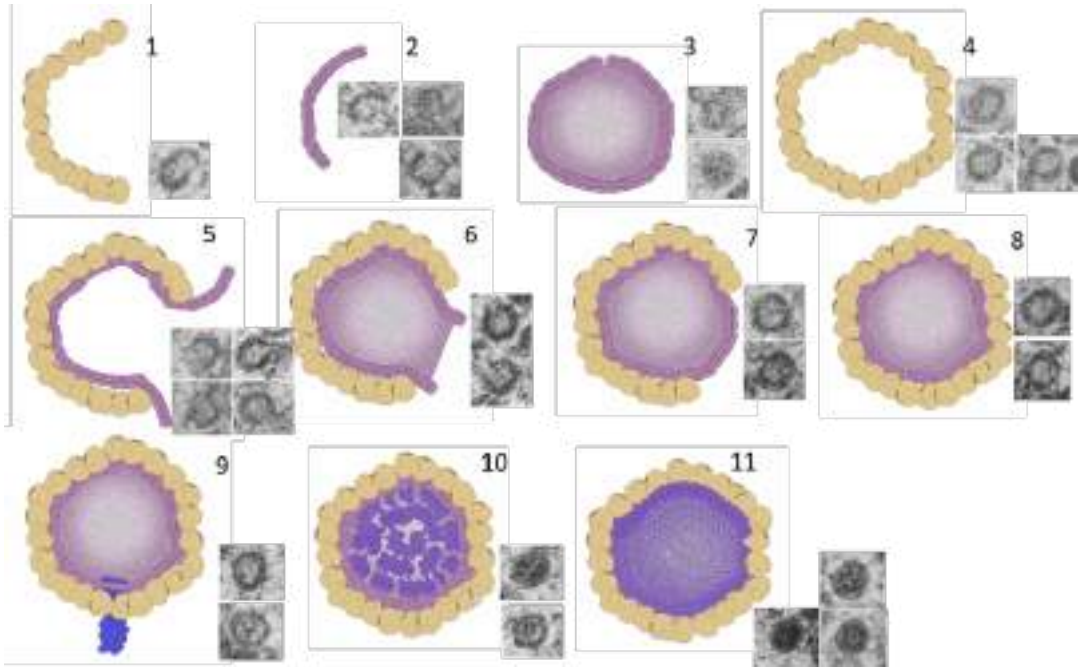
Adenovirus Assembly



Condezo and San Martín. 2017 PLoS Pathog 27

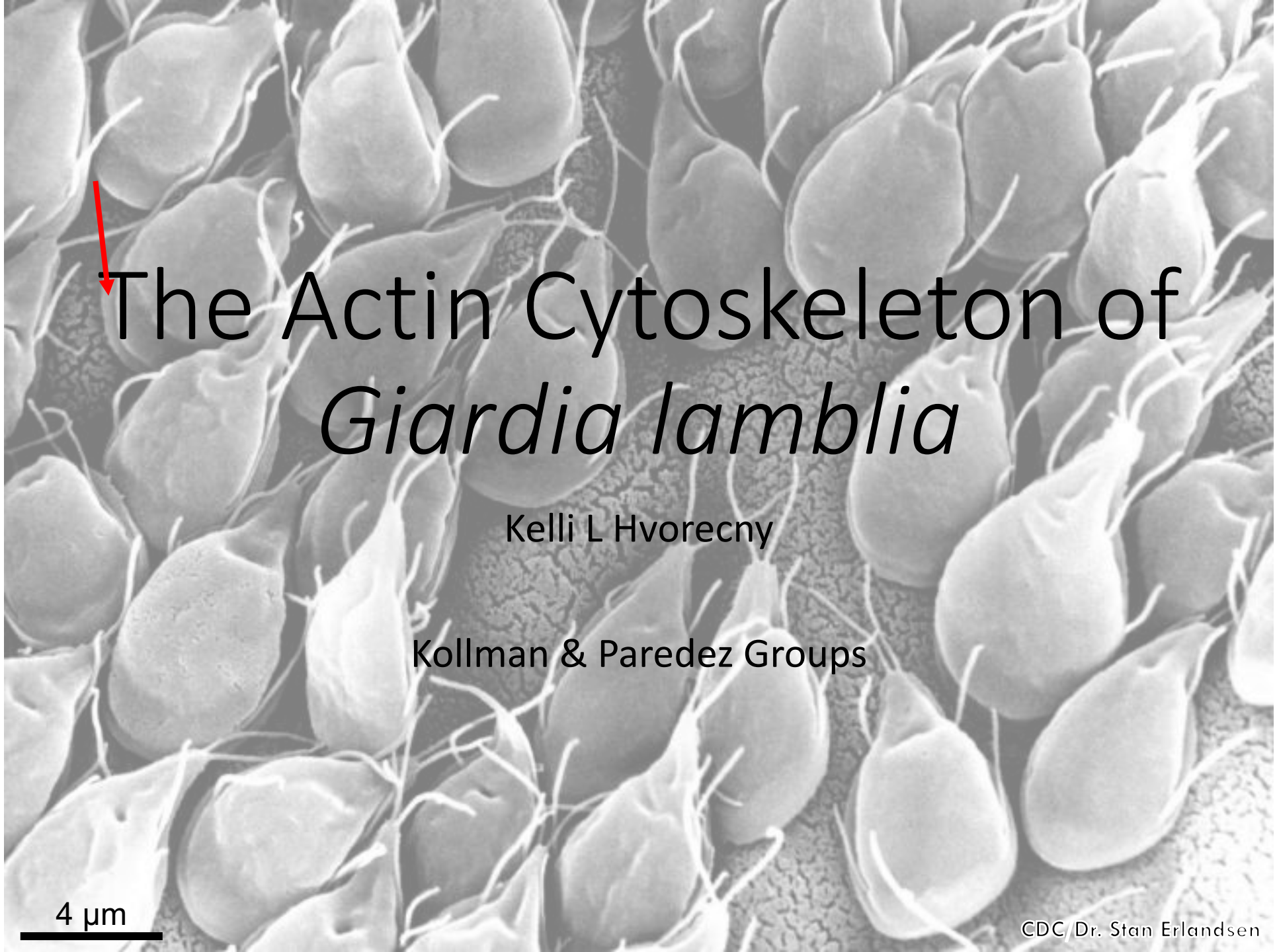
TsV-N1 Assembly





Kelli Hvorecny
University of Washington

Divergent Actin and a Lamellipodium-Like Structure in Giardia

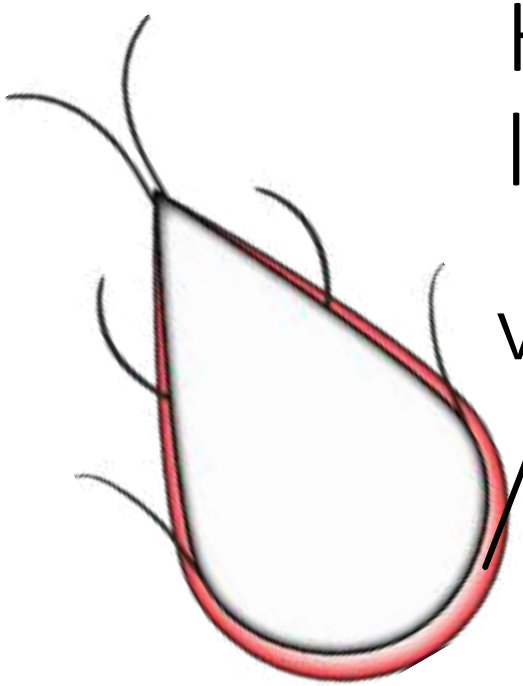


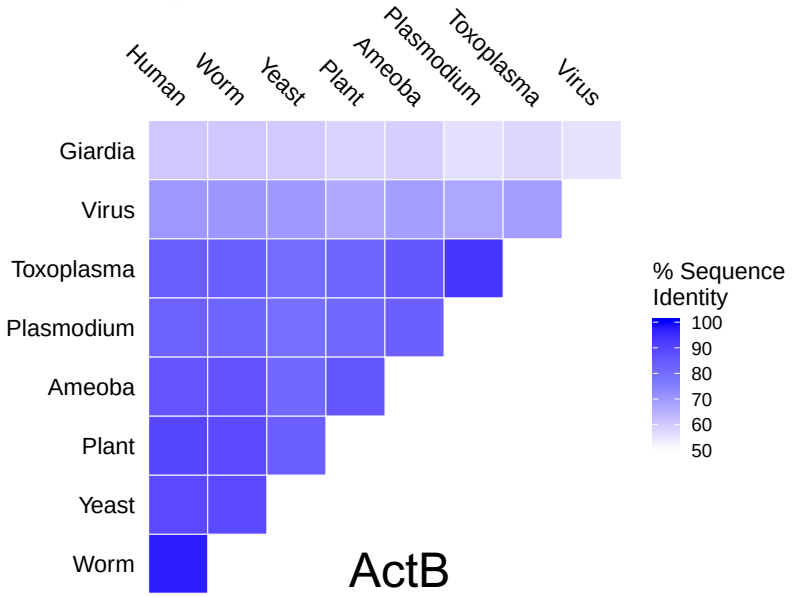
The Actin Cytoskeleton of *Giardia lamblia*

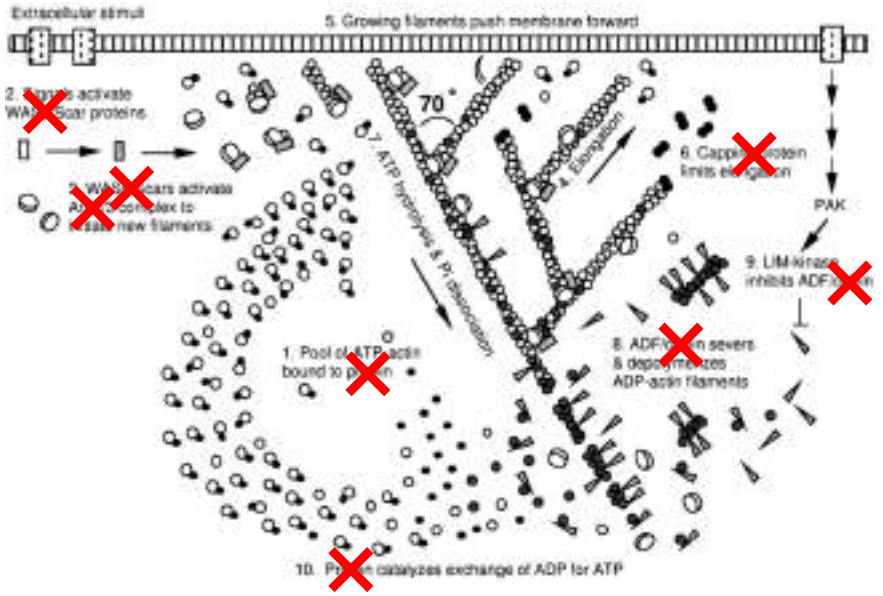
Kelli L Hvorecny

Kollman & Paredez Groups

How does *Giardia* form its lamellipodium-like structure?







Phalloidin

Cytochalasin D

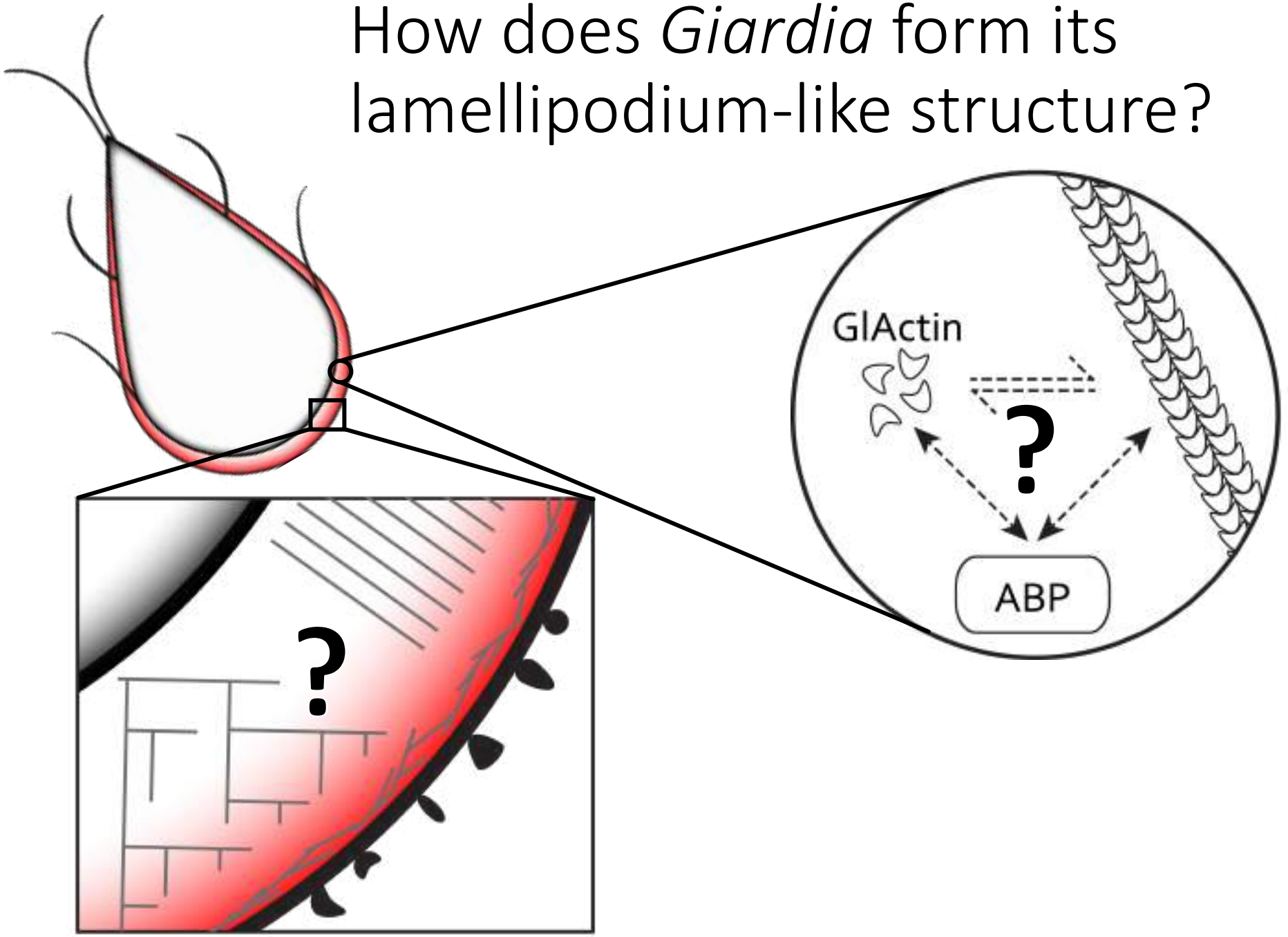
Latrunculin B

Morrison et al. 2007, *Science*.

Pollard. 2000; *Annu. Rev. Biophys. Biomol. Struct.*

Paredez et al. 2011 *PNAS*

How does *Giardia* form its lamellipodium-like structure?



Many thanks!

Justin Kollman

Joel Quispe

Eric Lynch

John Calise

Anika Burrell

Jesse Hansen

Andy Cai

Kenzee Hargett

Rachel Klevit

Harry Higgs

David Kovar

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UW Office of Postdoctoral Affairs

NIH NIAID AI145111

Alex Paredez

Bill Hardin

Elizabeth Thomas

Han-Wei Shih

Melissa Steele-Ogus

Germain Alas

Josh Vaughan

Aaron Halpern

Nate Sniadecki

Nikita Taparia

Pavla Tumova

Nadav Elad
Weizmann Institute

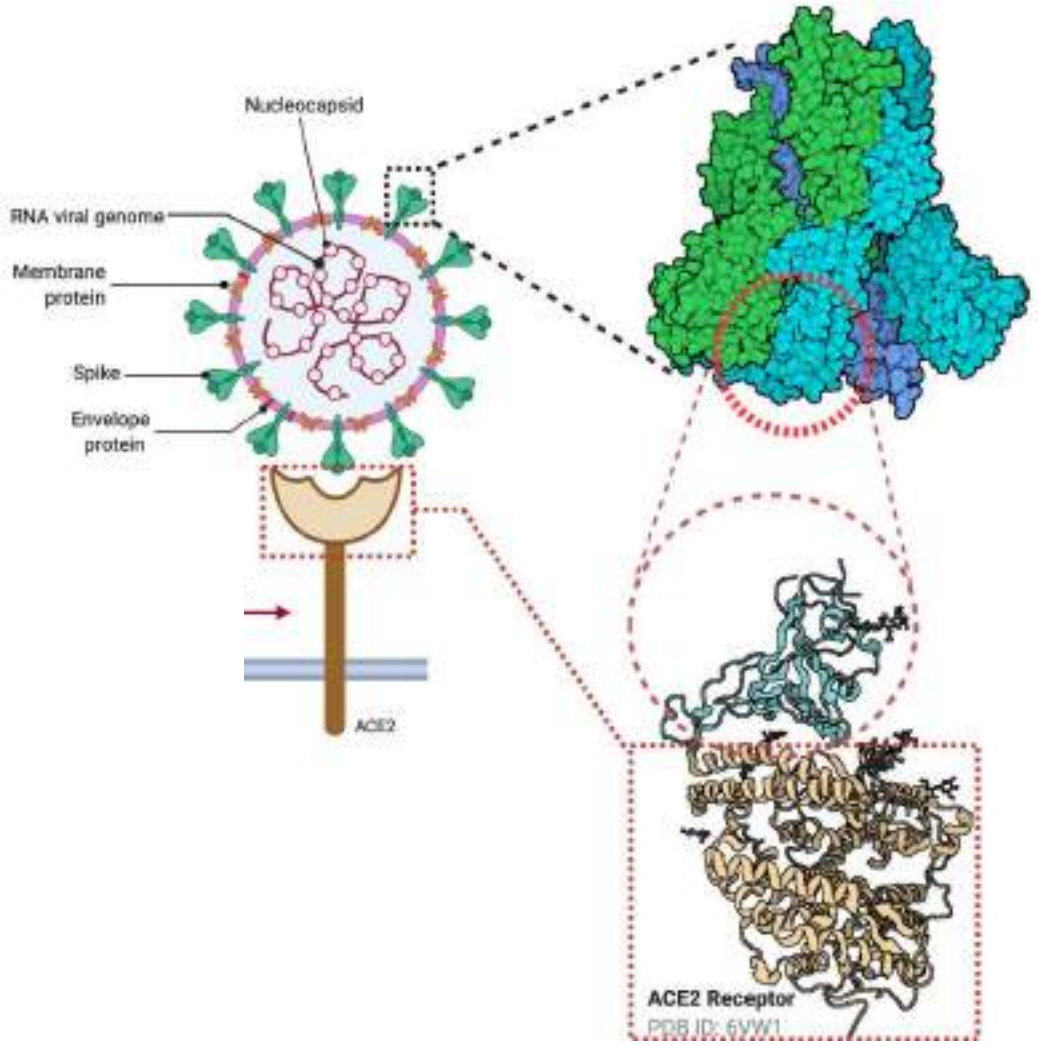
Structure of SARS-CoV-2 RBD baring contagious mutation in complex with ACE2

Structure of SARS-CoV-2 RBD baring contagious mutation in complex with ACE2

Nadav Elad

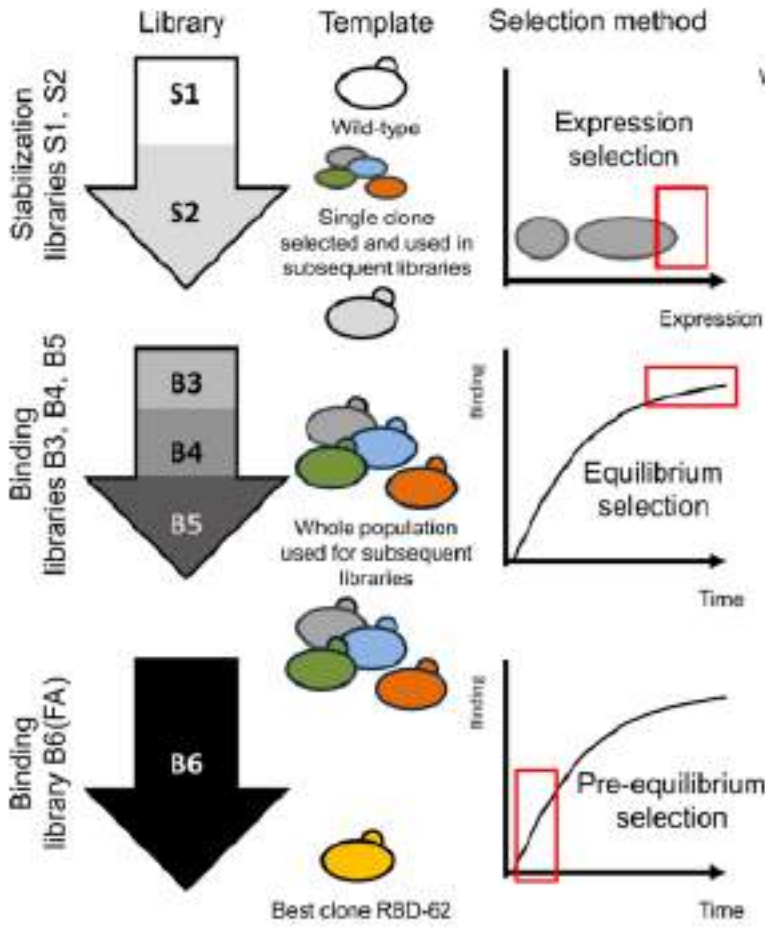
Electron Microscopy Unit

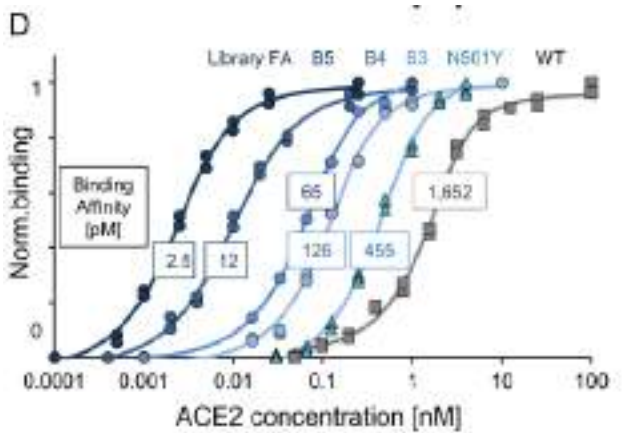
Weizmann Institute

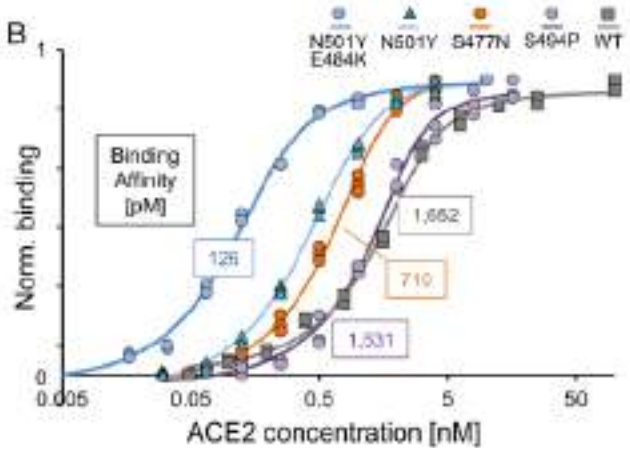


Wiese et al (2020) Molecules in pathogenesis

In vitro evolution of SARS-CoV-2 RBD

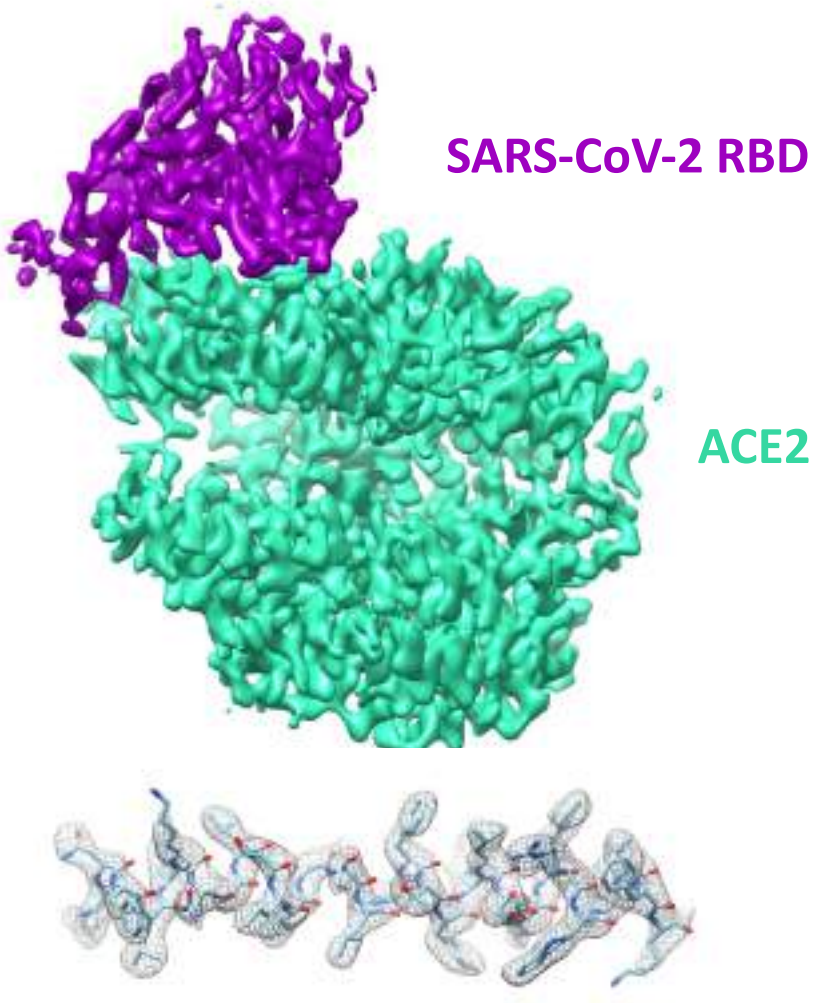


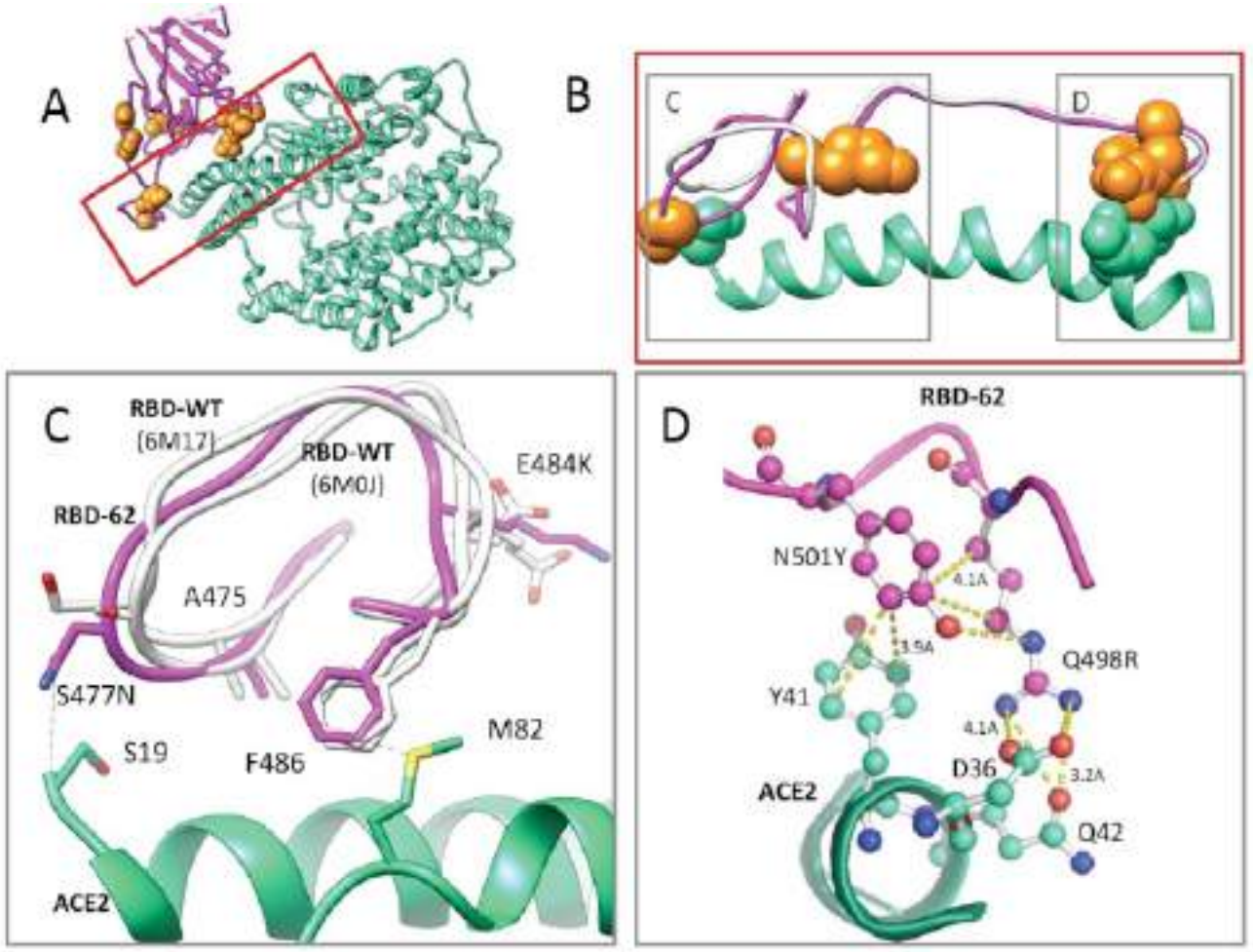




Jiří Zahradník, …, Orly Dym, Nadav Elad, Gideon Schreiber. **SARS-CoV-2 RBD *in vitro* evolution follows contagious mutation spread, yet generates an able infection inhibitor.** *https://www.**biorxiv**.org/content/10.1101/2021.01.06.425392v3*

Structure of mutated SARS-CoV-2 RBD – ACE2





Jiří Zahradník, …, Orly Dym, Nadav Elad, Gideon Schreiber. **SARS-CoV-2 RBD *in vitro* evolution follows contagious mutation spread, yet generates an able infection inhibitor**. *https://www.**biorxiv**.org/content/10.1101/2021.01.06.425392v3*

Mark Kreutzberger
University of Virginia

Visualizing compressional distortions of the supercoiled bacterial flagellar filament in a thin layer of ice

Visualizing compressional distortions of the supercoiled bacterial flagellar filament in a thin layer of ice

Mark Kreutzberger

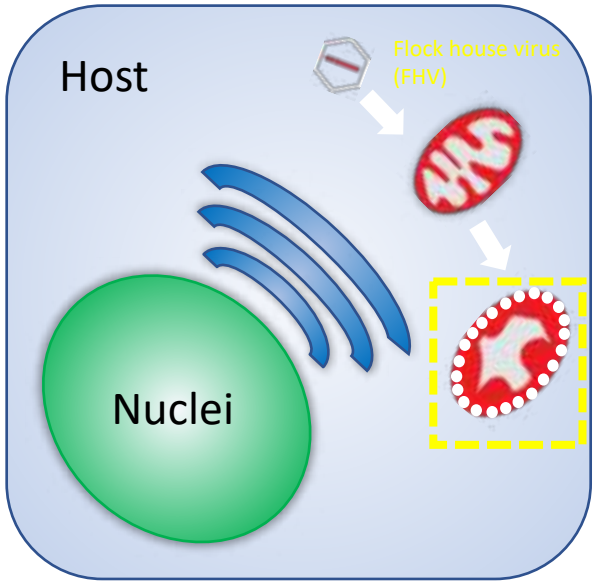
Egelman Lab

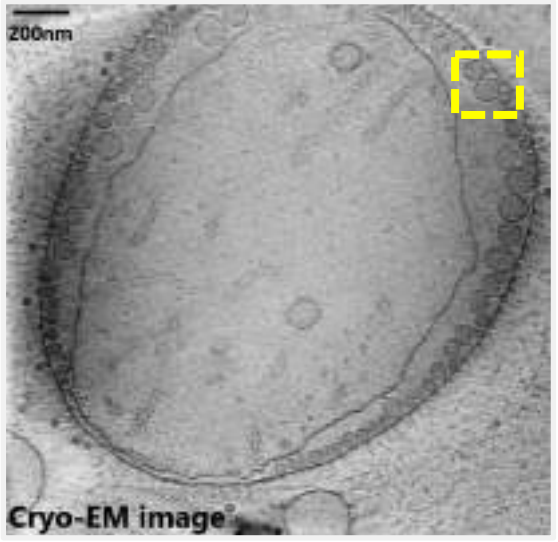
University of Virginia

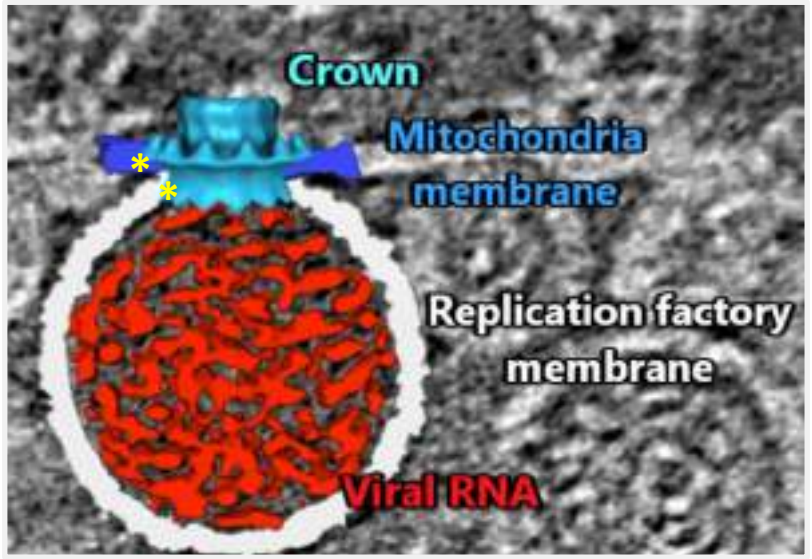
Hong Zhan
University of Wisconsin-Madison

Deciphering the nano-machinery of RNA viral replication using Cryo-EM subtomogram averaging

Deciphering the Nano-machinery of RNA Viral Replication Using Cryo-EM Subtomogram Averaging

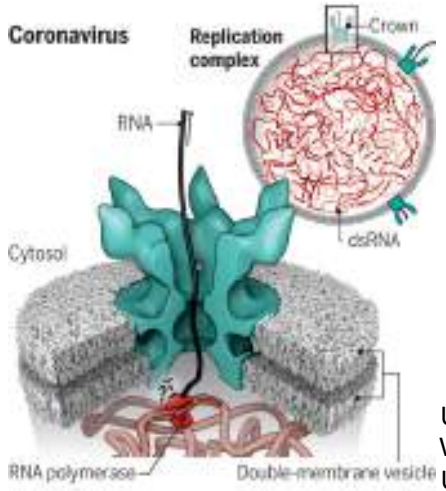


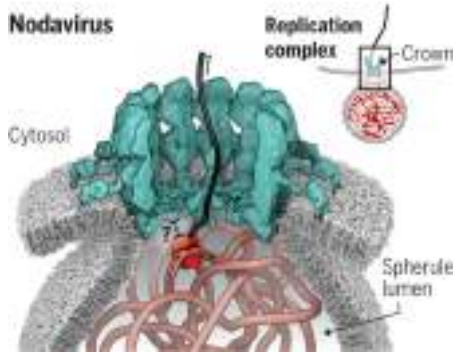




Ertel *et al*. eLife 2017

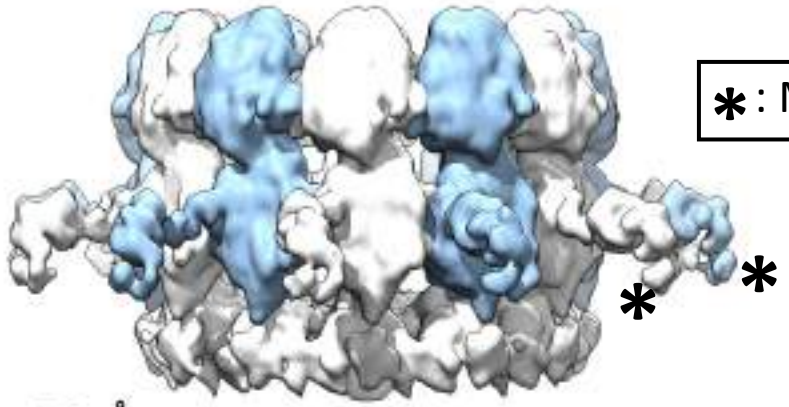
Similar replication ultrastructure feature in RNA virus family





Unchwaniwala and Ahlquist. Science 2020
Wolff *et al*. Science 2020
Unchwaniwala, Zhan *et al*. PNAS 2020

FHV "Crown"



50 Å

4640 crowns from 59 tomograms
Unchwaniwala, Zhan *et al*. PNAS 2020

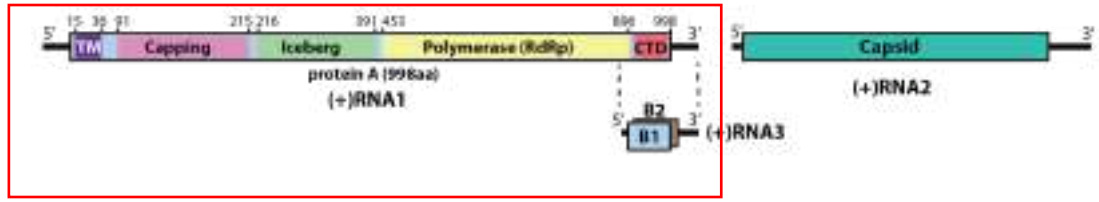
*: Membrane contact points

Hong Zhan

NCCAT Cryo-EM workshop
April 13th, 2021

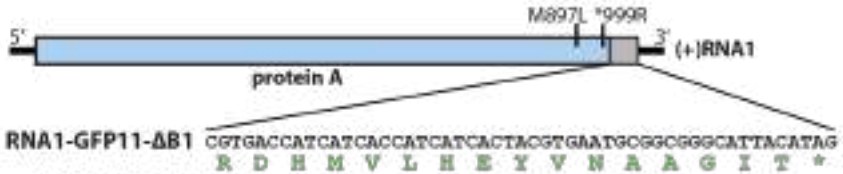
Site-Specific Labeling of FHV Protein A C-Terminus

FHV genome

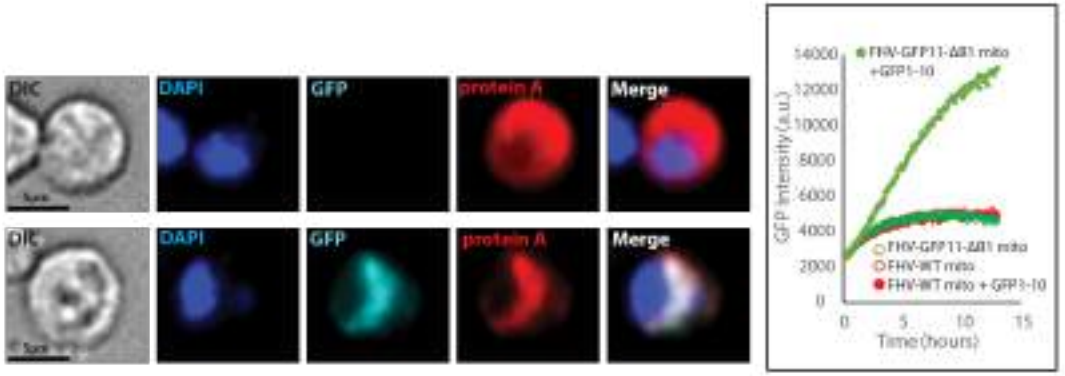


Replication process

FHV protein A C-terminus fused with GFP-11 tag

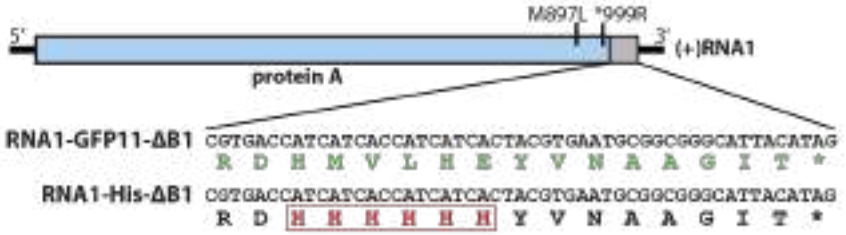


FHV protein A C-ter is accessible to the cytoplasm

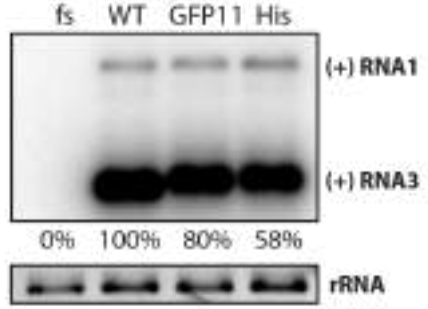


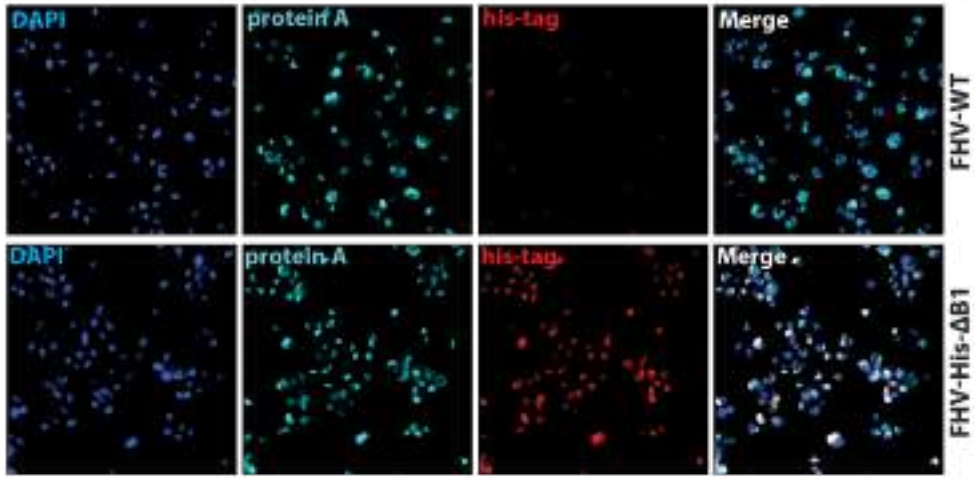
Unchwaniwala, Zhan *et al*. PNAS 2020

FHV protein A C-terminus fused with His tag for EM labeling



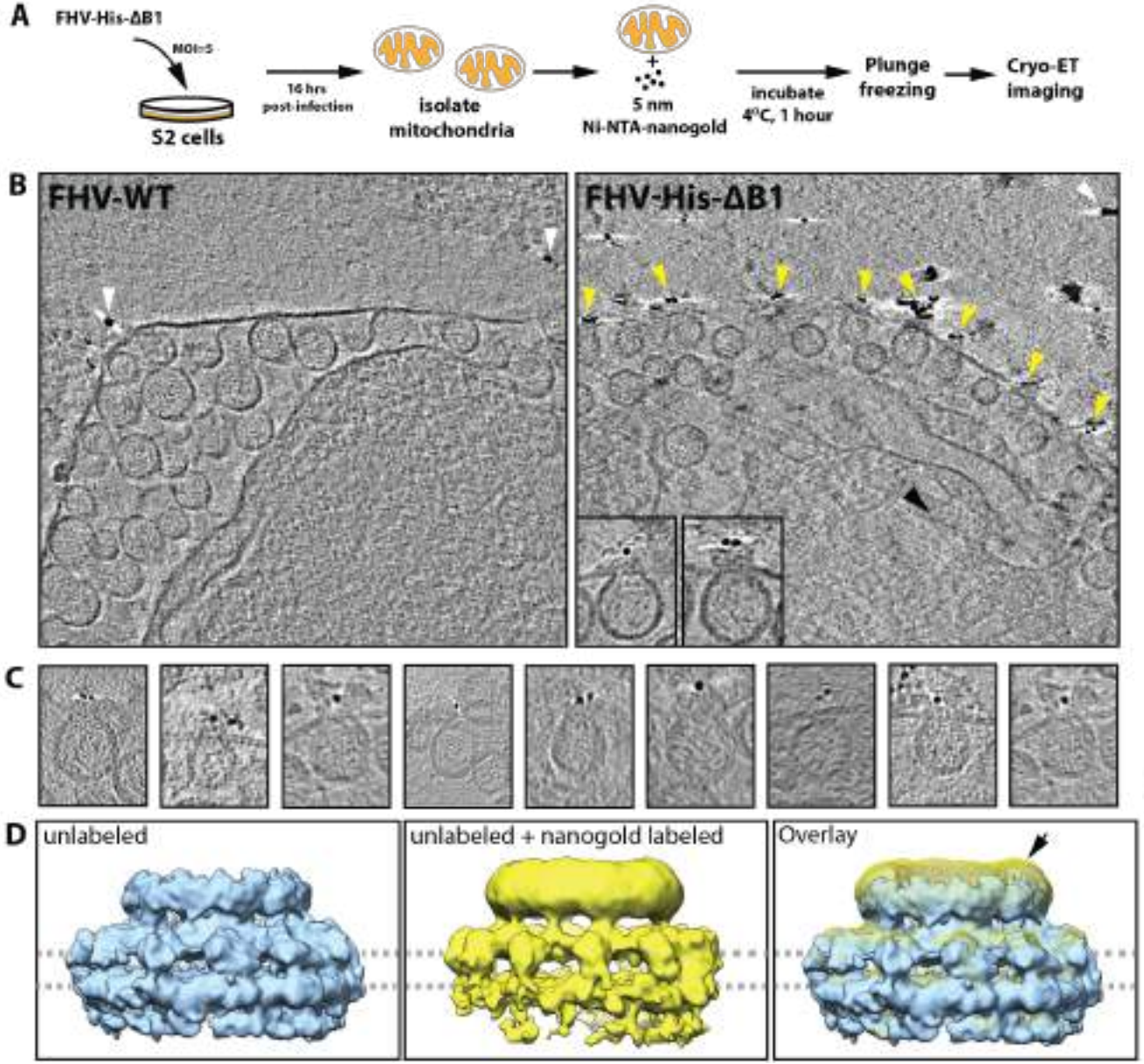
FHV protein A C-terminus tag insertion does not impair replication process



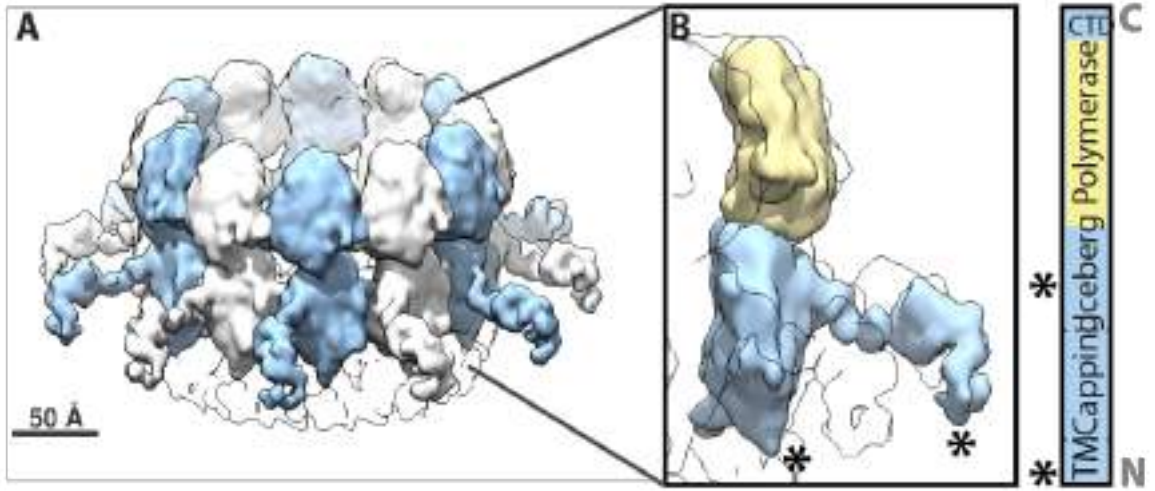


Unchwaniwala, Zhan *et al*. PNAS 2020

FHV Protein A C-Terminus is located at Crown Apex

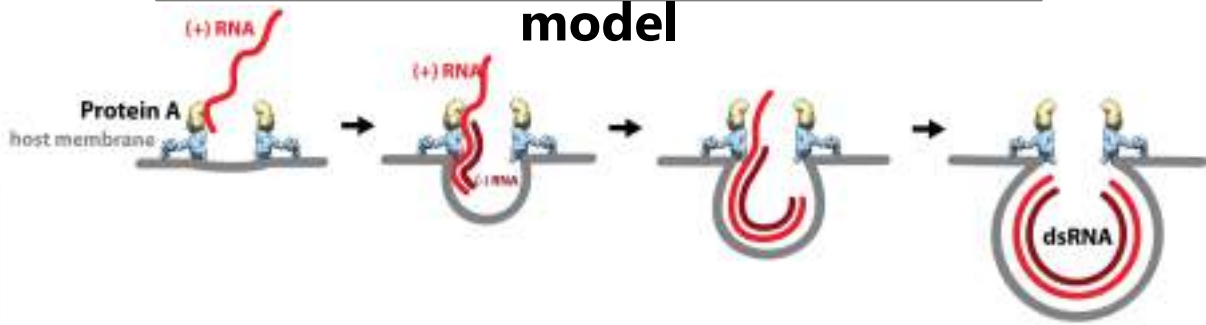


Unchwaniwala, Zhan *et al*. PNAS 2020



Unchwaniwala, Zhan *et al*. PNAS 2020

Replication/dsRNA synthesis model

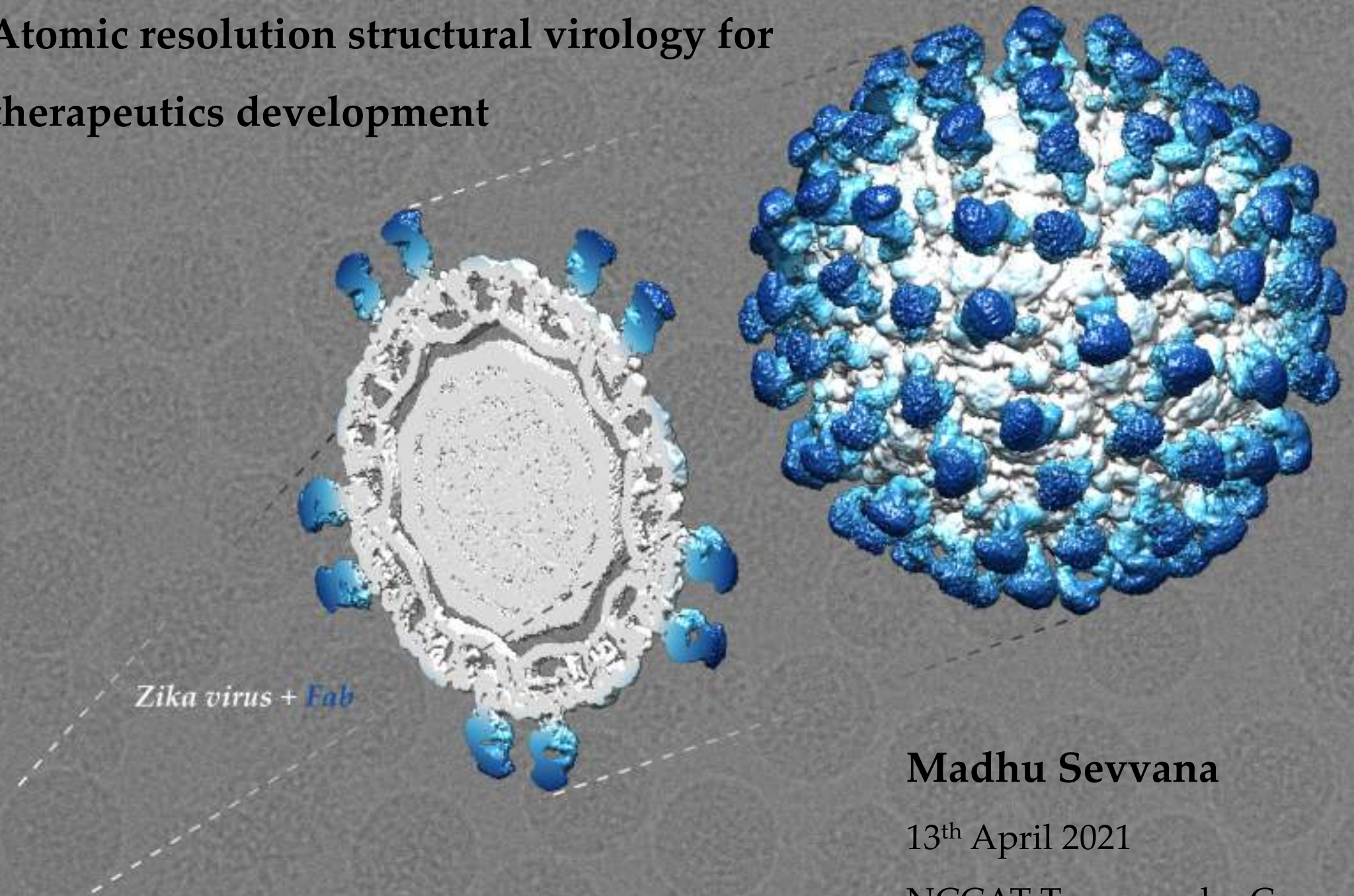


increase in vesicle size during dsRNA template synthesis

Madhumati Sevvana
Purdue University

Towards high resolution structural virology for therapeutics development

Atomic resolution structural virology for therapeutics development



Madhu Sevvana

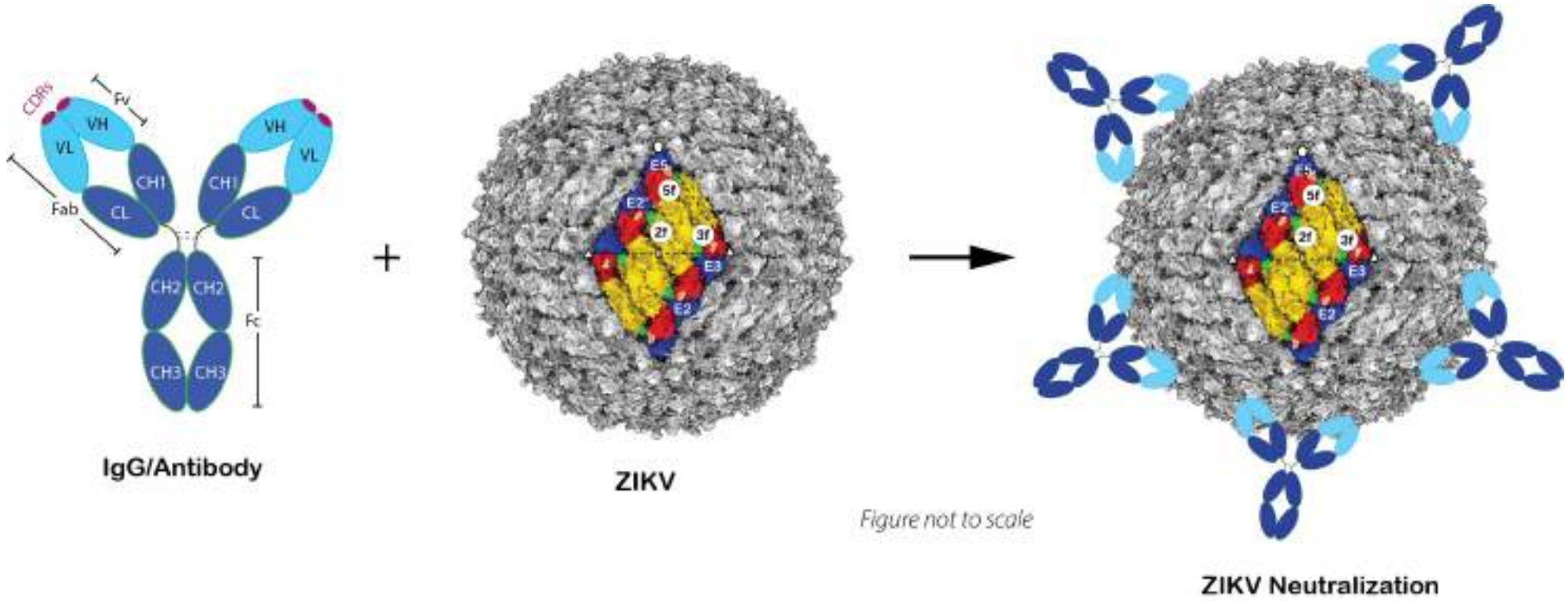
13th April 2021

NCCAT Tomography Course

THE KUHN LAB

PURDUE UNIVERSITY

Study antibody mediated neutralization of viruses for structure-based therapeutics and vaccine development



Why cryo-ET?

- Asymmetric complexes of icosahedral viruses and Abs
- 3D structure of pleomorphic viruses in complex with Abs

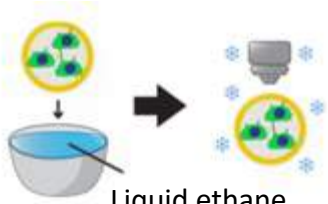
Jae Yang
University of Wisconsin-Madison

Studying in-situ viral infection with multi-modal cryogenic correlative FLM-FIB/SEM-ET and CorRelator

Exploring *in-situ* viral infection with cryogenic correlative FLM-FIB/SEM-ET and CorRelator

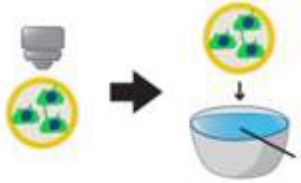
Jae Yang
Wright Laboratory & Midwest Center for Cryo-Electron Tomography (MCCET),
Department of Biochemistry, University of Wisconsin-Madison

Plunge freezing -> cryo-FLM

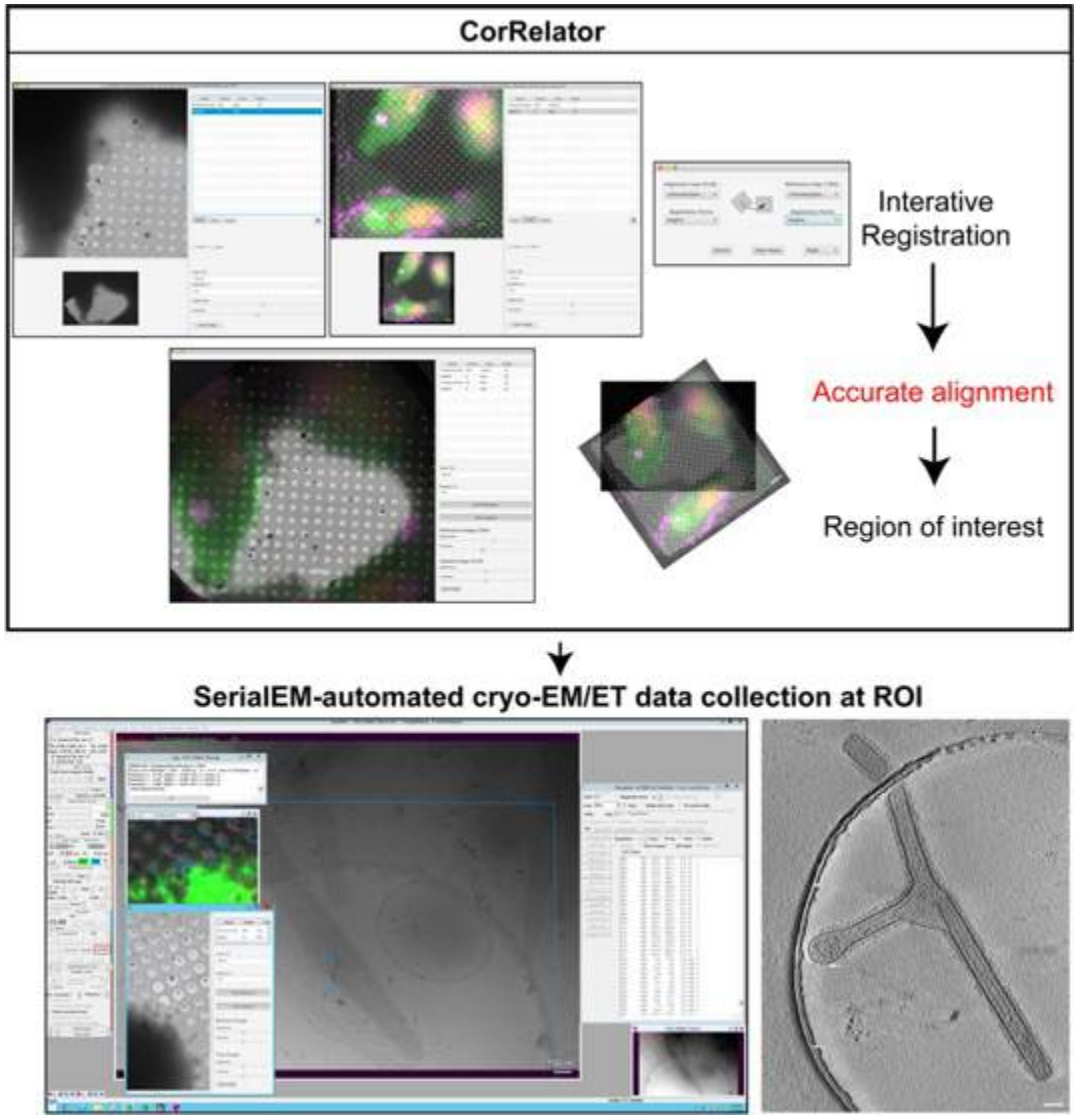


Liquid ethane freezing

RT FLM -> Plunge freezing



Liquid ethane freezing

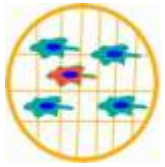


CorRelator supports:

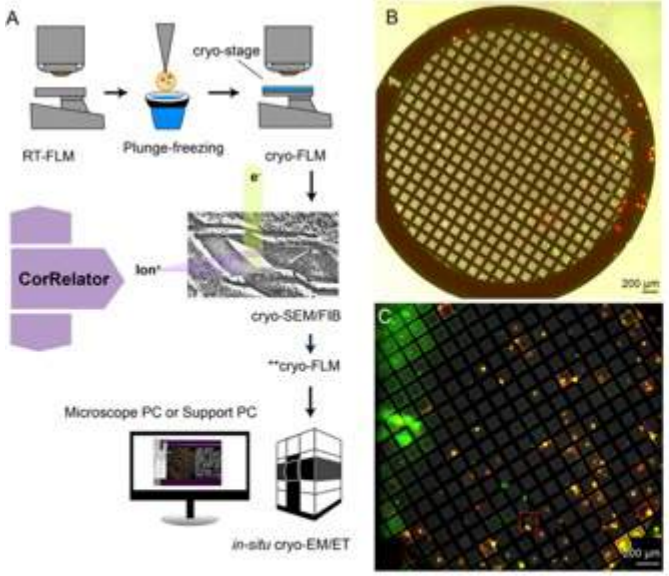
- Cross-platform & system-independent correlations
- Accurate and flexible on-the-fly (with SerialEM) and post-acquisition correlation
- Combination of algorithmic and human interactive registration
- Intuitive, and user-friendly application
- Free and source code available at https://github.com/wright-cemrc-projects/corr.

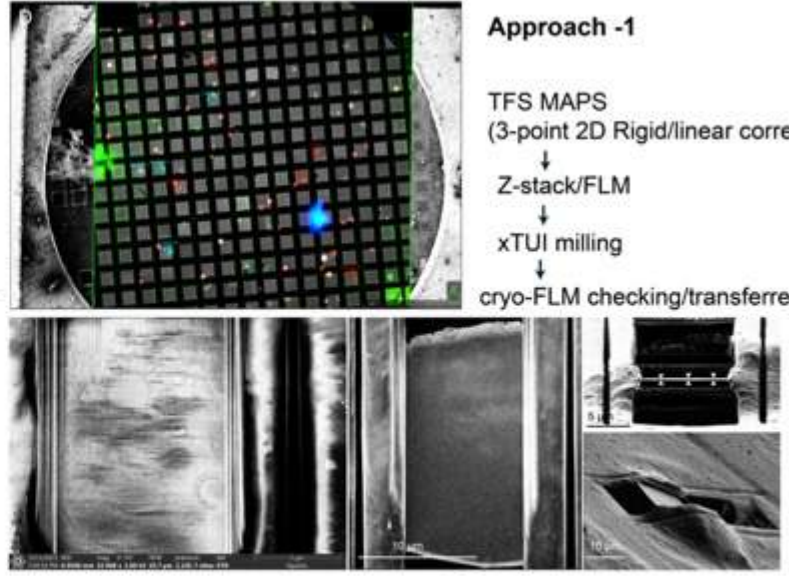
J Struct Biol, 2021, Yang J. & Larson M., et al.
https://github.com/wright-cemrc-projects/corr.

Multi-modal correlative approaches to study respiratory syncytial virus infection in HeLa cells



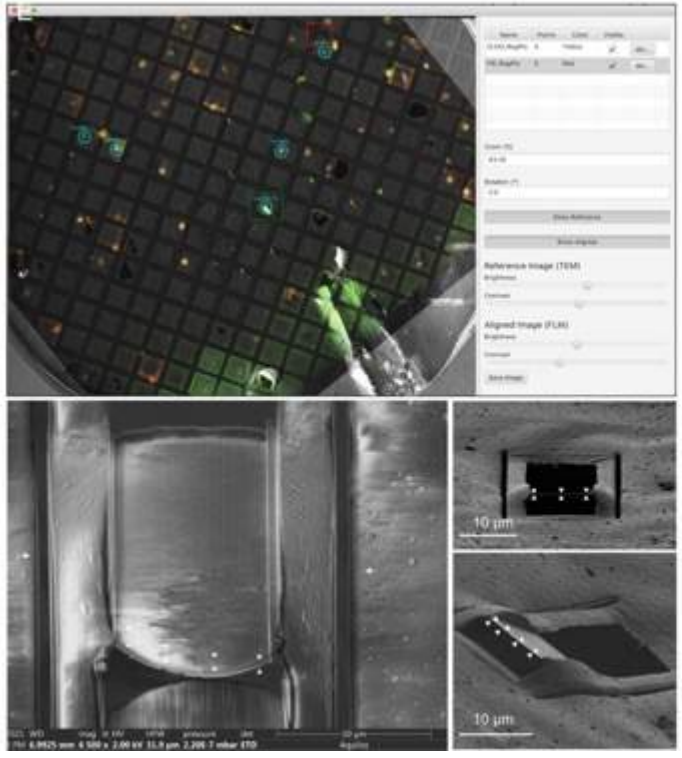
HeLa cells infected by respiratory syncytial virus (red fluorescent signal), live-cell labeling of mitochondria and or microtubules (green fluorescent signal)





Approach -1

TFS MAPS
(3-point 2D Rigid/linear correlation)
↓
Z-stack/FLM
↓
xTUI milling
↓
cryo-FLM checking/transferred out



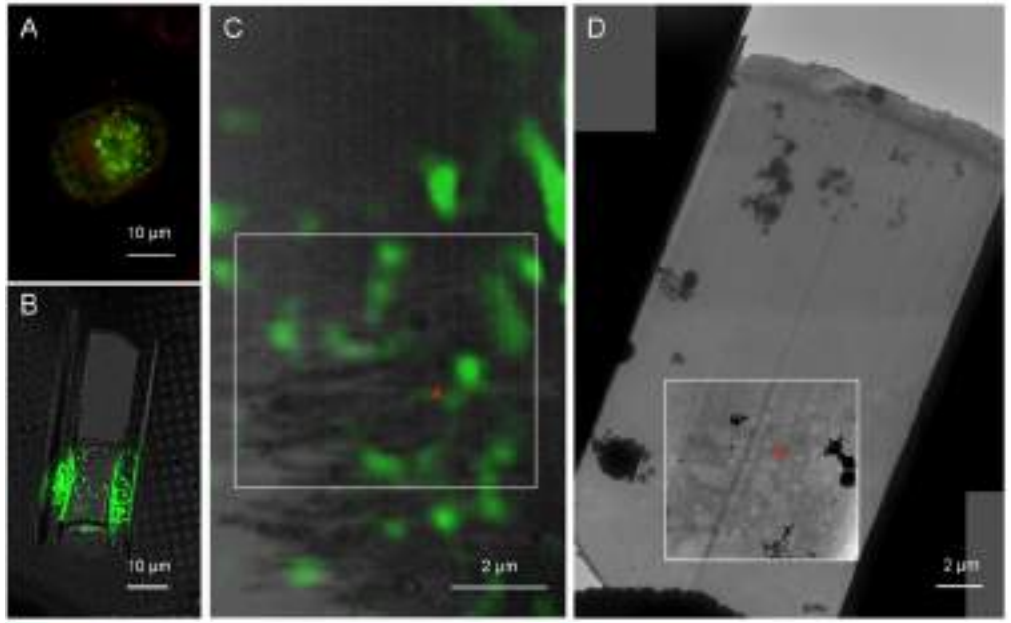
Approach -2

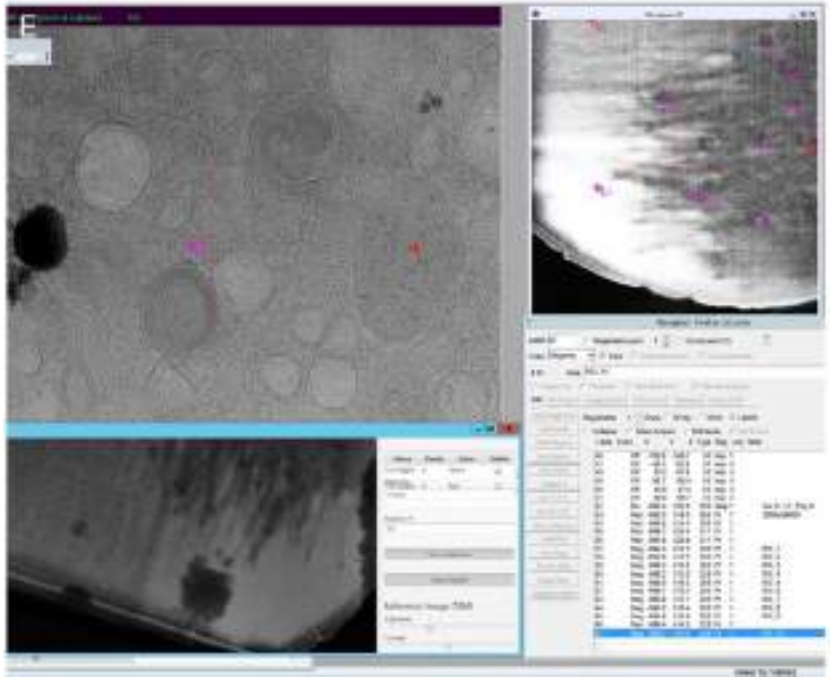
CorRelator
(Multiple-point 2D Rigid and non-linear correlation)
↓
Z-stack/FLM
↓
xTUI milling
↓
cryo-FLM checking

↓
3D FLM-SEM correlation
↓
SEM-FIB correlation
↓
Automated xTUI milling

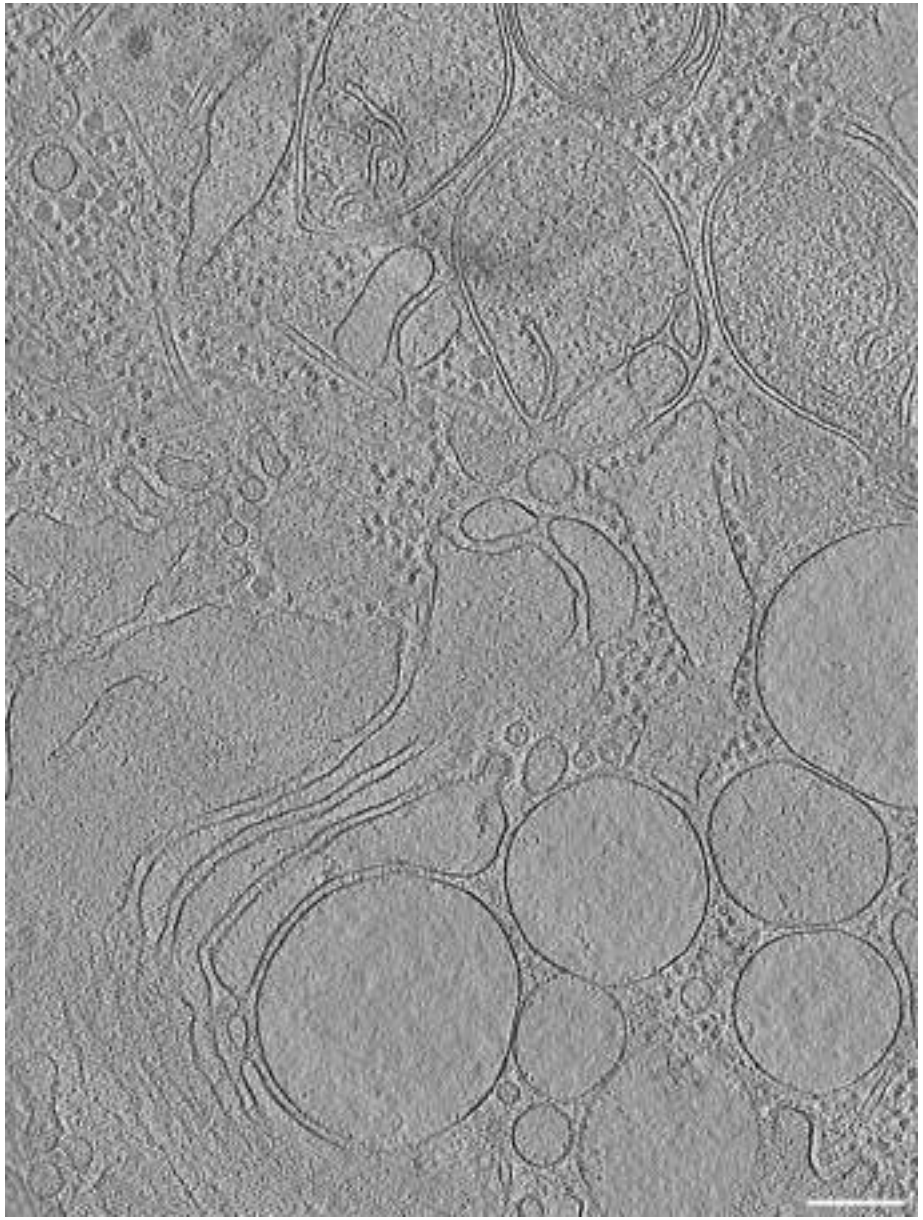
CorRelator
(active development)

Cryo-ET of regions of interest (ROIs) in infected cells





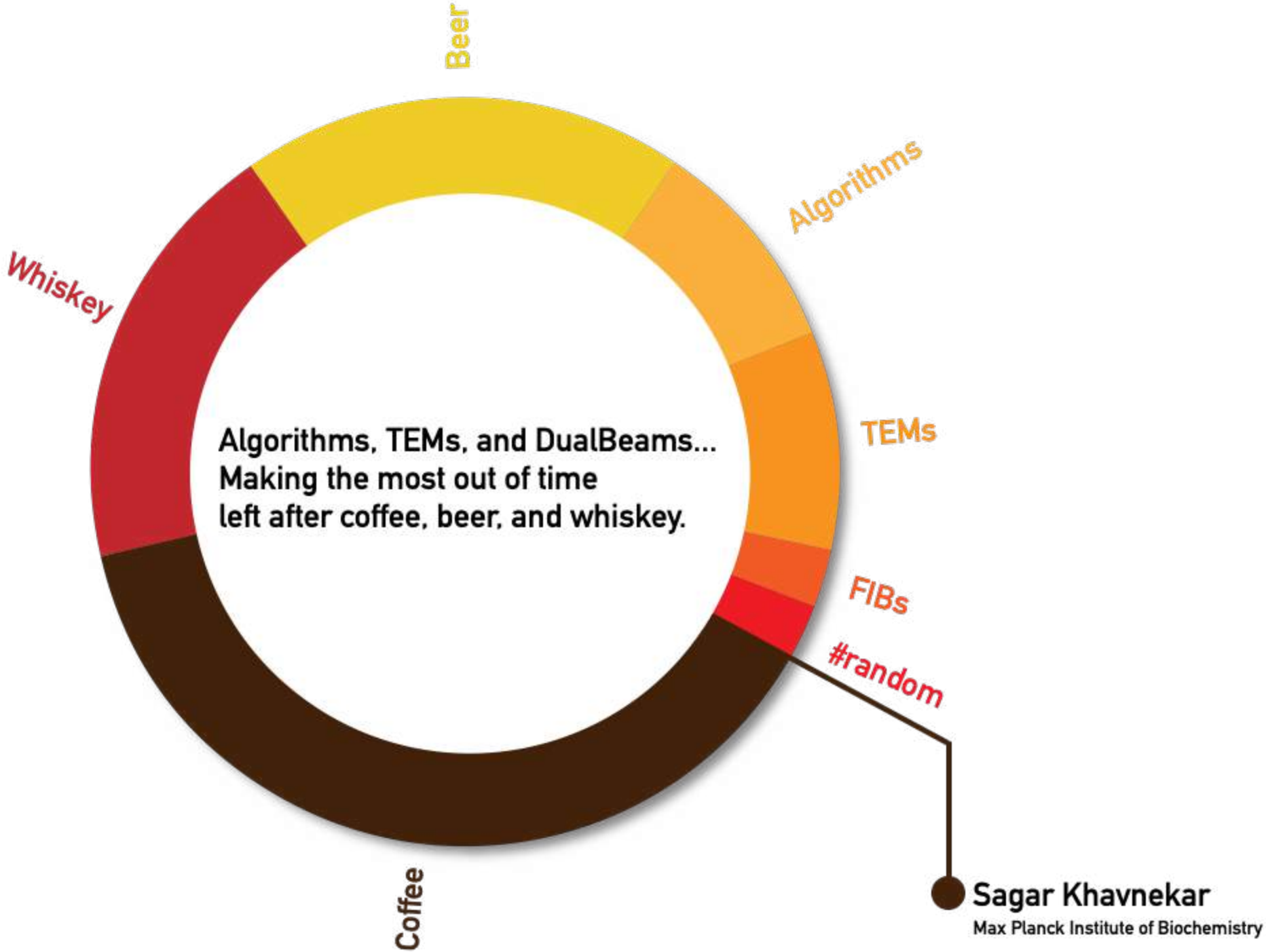
Mitochondria (green)
in infected (X) and uninfected, healthy.
(X) HeLa cells

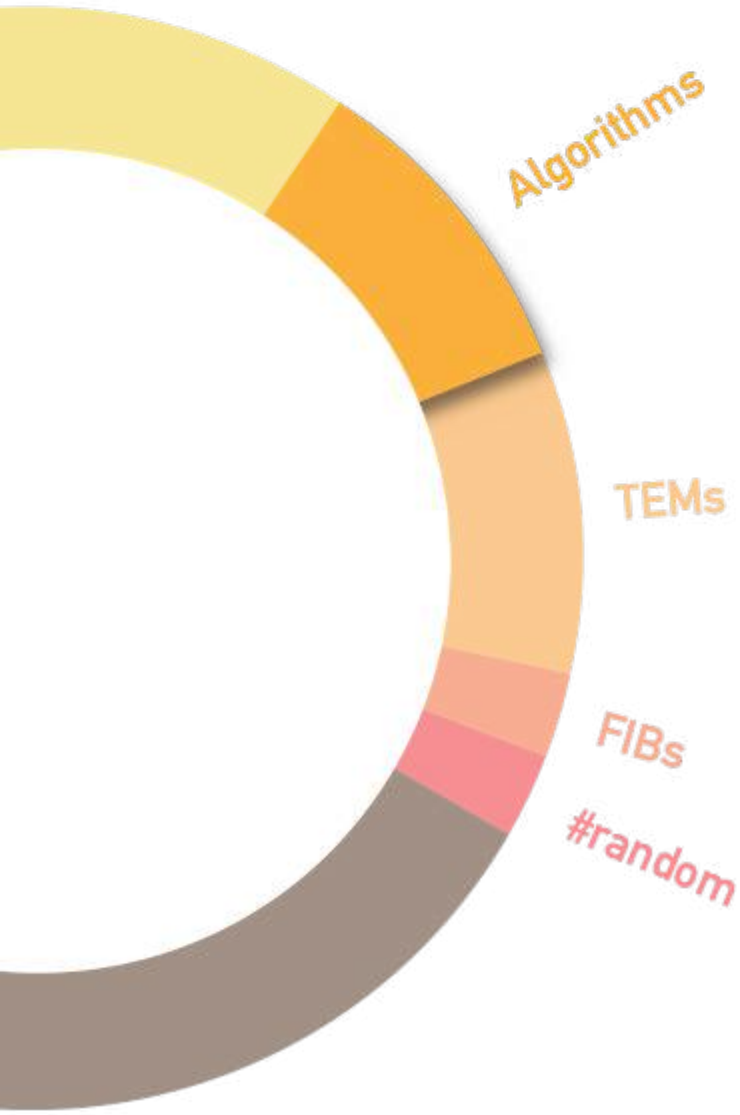


Tomogram slice (thickness of 30 nm) of ROIs in uninfected, healthy HeLa cells,
Scale bar = 200 nm

Sagar Khavnekar
Max Planck Institute of Biochemistry

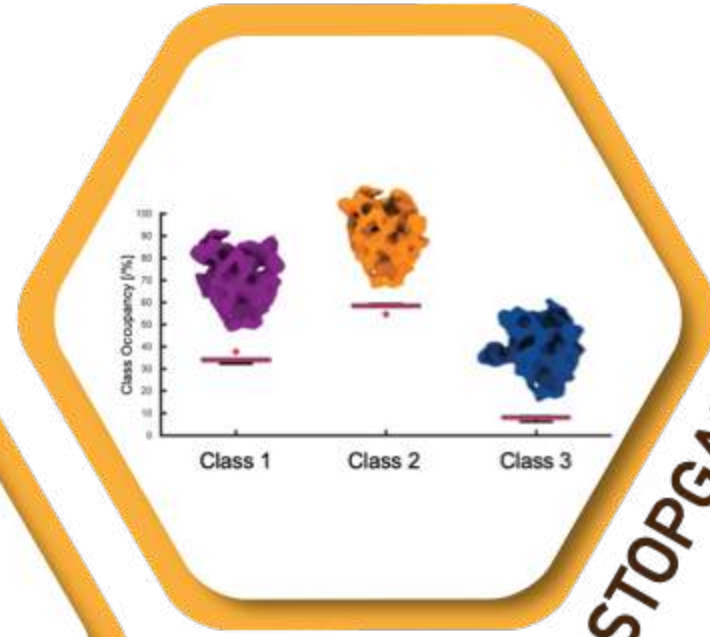
Algorithms, TEMs, and DualBeams... Making the most out of time left after coffee, beer, and whisky.


Beer
Algorithms
TEMs
FIBs
#random
Coffee
Whiskey
Algorithms, TEMs, and DualBeams...
Making the most out of time
left after coffee, beer, and whiskey.
Sagar Khavnekar
Max Planck Institute of Biochemistry

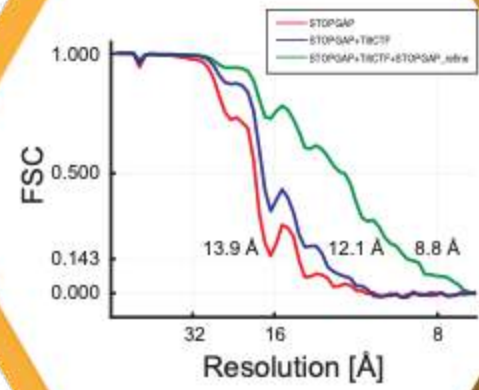


TOMOMAN

Faster, Automated preprocessing, and data management with TOMOgram MANager

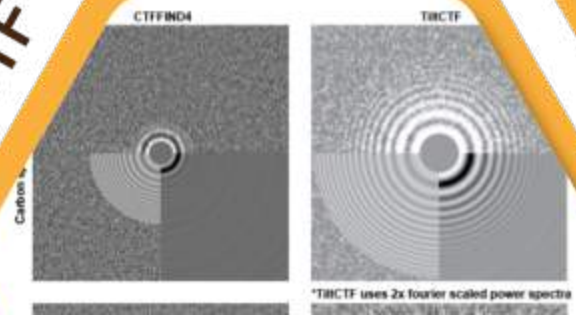


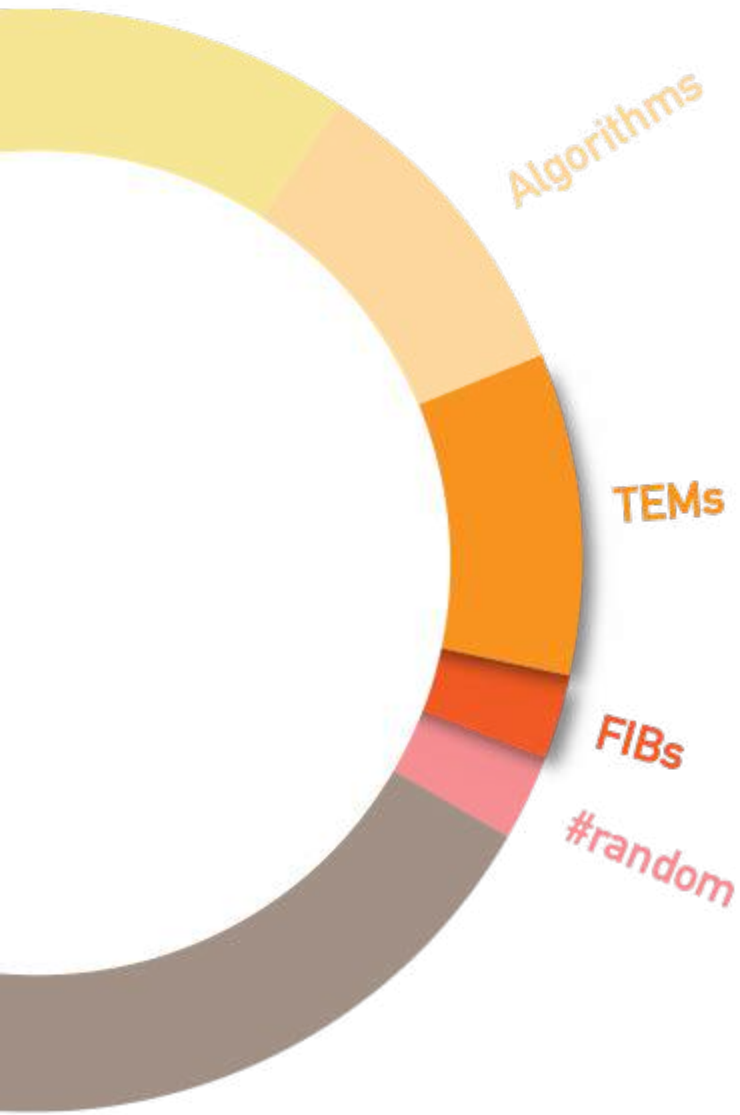
STOPGAP

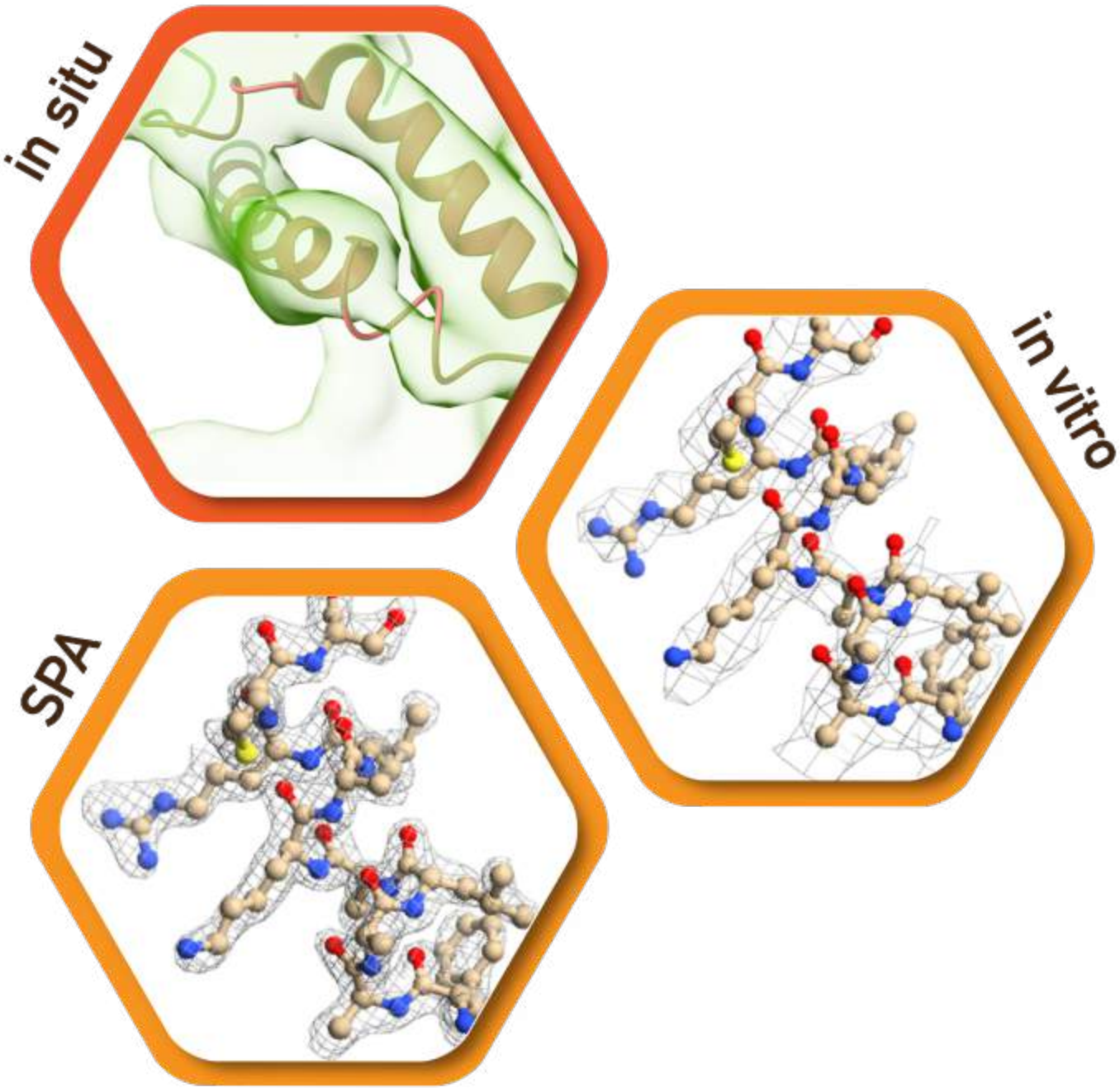


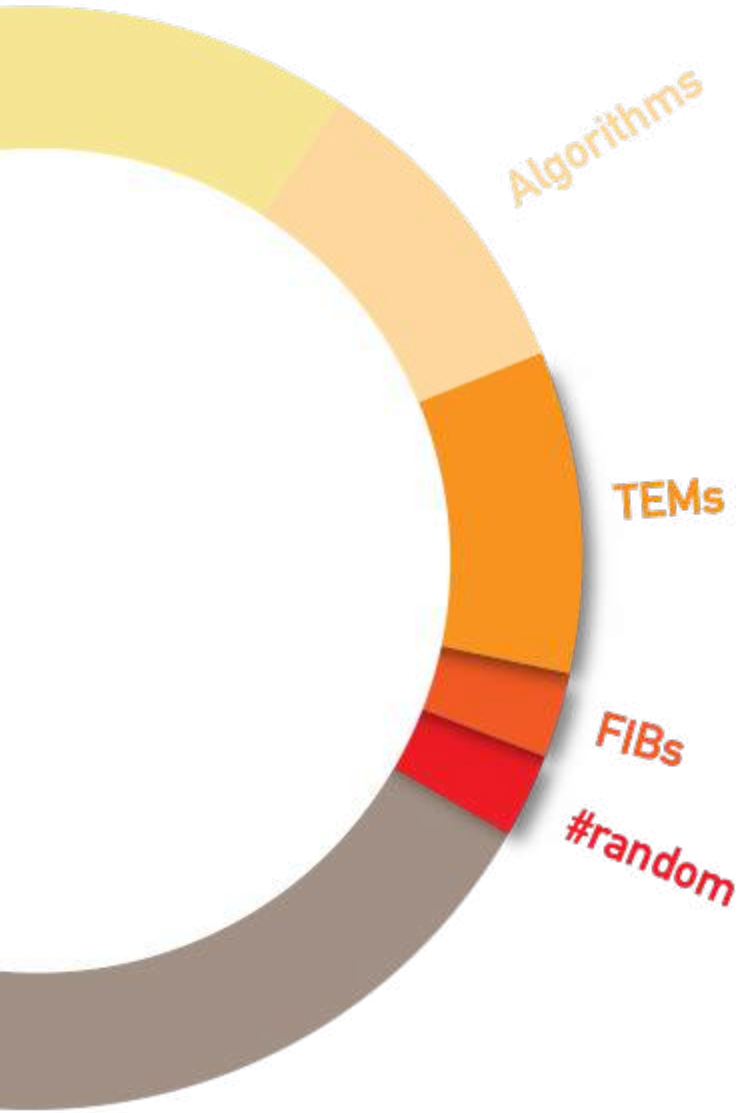
refine

tiltCTF



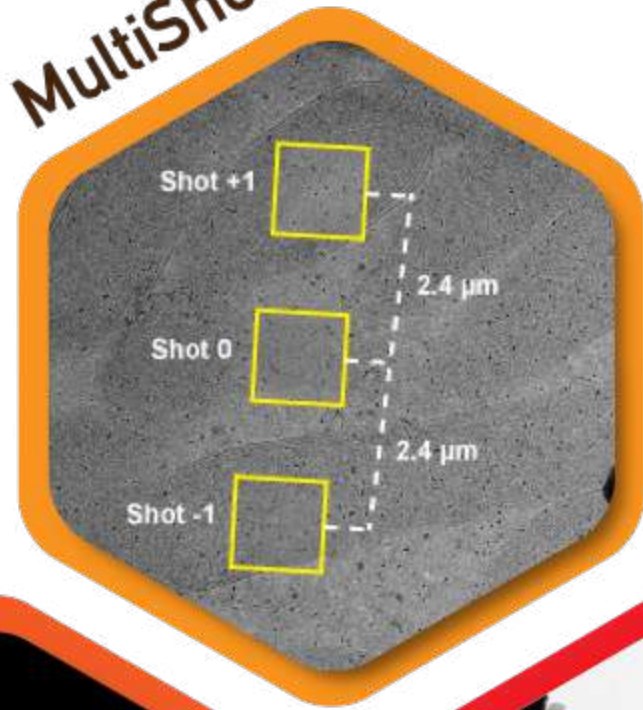

Algorithms
TEMs
FIBs
#random


in situ
in vitro
SPA

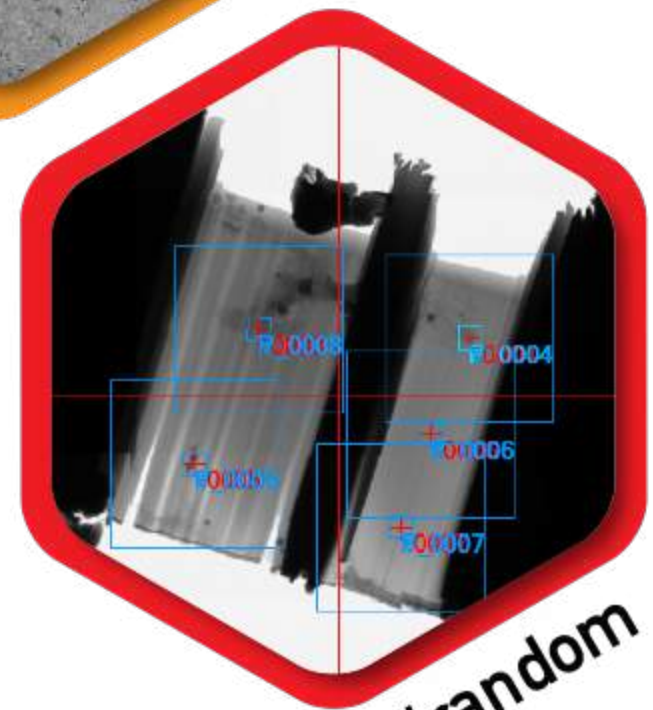


@cryosagar

MultiShot



Waffles



#random